Ellagic Acid Attenuated High Blood Pressure, but not Regresses Cardiac Remodelling in L-NAME-induced Hypertensive Rat
แอลลาจิคแอซิดลดความดันเลือดสูง แต่ไม่ลดการปรับเปลี่ยนโครงสร้างหัวใจในหนูที่ทำให้เกิดความดันเลือดสูงด้วย L-NAME

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ABSTRACT

Ellagic acid (EA) is a naturally polyphenolic compound, it has been shown to be a potent benefit for antioxidant property. The aim of this study was to investigate the preventive effect of EA on blood pressure, cardiac wall dimension in nitric oxide-deficient hypertensive rats. Male Sprague-Dawley rats were given N-nitro-L-arginine methyl ester (L-NAME) in drinking water to induce hypertension, and simultaneously treated with EA (L-NAME+EA group), or a vehicle (L-NAME). Age-matched rats served as a control group. Systolic blood pressure, body weight, ventricular weight and heart weight were determined. Heart wall thickness and cross-sectional areas were evaluated. Systolic blood pressure in L-NAME+EA rats was significantly lower than those in L-NAME ($p<0.05$). EA did not affect on the relative heart weight and ventricular cross-section area. In conclusion, EA has antihypertensive property in nitric oxide deficiency model but did not effect to a remodelling of the cardiac wall.

บทคัดย่อ

แอลลาจิคแอซิดคือสารประกอบฟีโนลิคซึ่งมีประสิทธิภาพดีในการต้านอนุมูลอิสระ วัตถุประสงค์ของการศึกษาในครั้งนี้เพื่อศึกษาผลการป้องกันของแอลลาจิคแอซิดต่อความดันเลือด ขนาดและน้ำหนักของหัวใจในหนูที่ทำให้เกิดความดันเลือดสูงจากภาวะพร่องไนตริกออกไซด์ หนูขาวเพศผู้ถูกเหนี่ยวนำให้เกิดความดันเลือดสูงโดยได้รับ N-nitro-L-arginine methyl ester (L-NAME) ในน้ำดื่มร่วมกับแอลลาจิคแอซิด (กลุ่ม L-NAME+EA) หรือ กลุ่มได้รับ L-NAME อย่างเดียว (L-NAME) และกลุ่มควบคุม โดยวัดค่าความดันเลือด น้ำหนักตัว น้ำหนักหัวใจห้องล่างและน้ำหนักของหัวใจทั้งหมด รวมทั้งศึกษาความหนา

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และพื้นที่หน้าตัดของหัวใจ พบว่าความดันเลือดซีสโตลิกในหนูกลุ่ม L-NAME+EA ต่ำกว่าความดันเลือดซีสโตลิกในหนูกลุ่ม L-NAME อย่างมีนัยสำคัญ (p<0.05) แอลลาจิคแอซิดไม่มีผลต่อน้ำหนักหัวใจ และพื้นที่หน้าตัดของผนังห้องล่างของหัวใจ โดยสรุปแอลลาจิคแอซิดมีฤทธิ์ต้านความดันเลือดสูงในภาวะพร่องไนตริกออกไซด์ แต่ไม่มีผลต่อการปรับเปลี่ยนโครงสร้างของผนังหัวใจ

Key Words : L-NAME, Ellagic acid, Ventricle, Hypertension
คำสำคัญ : แอลเนม แอลลาจิคแอซิด หัวใจห้องล่าง ความดันเลือดสูง

Introduction

Hypertension is one of the most important public health problems worldwide and it is a common cause of cardiovascular disease including ischemic heart disease and finally cardiac failure [3, 4].

Nitric oxide (NO) plays an important role in maintaining cardiovascular homeostasis, fibrinolysis, platelet and leukocyte interactions with the arterial wall, regulation of vascular tone, proliferation of vascular smooth muscle cells, and homeostasis of blood pressure. Disturbances in NO bioavailability have been linked to cause endothelial dysfunction, leading to increased susceptibility to atherosclerotic lesion progression, hypertension, diabetes mellitus, hypercholesterolemia, thrombosis and stroke [2, 12, 14].

N-nitro-L-arginine methyl ester (L-NAME) is a nonspecific inhibitor of all three NO synthase (NOS) isoforms (neuronal, inducible, and endothelial NOS) [8, 17]. It has been reported that L-NAME administration can cause increased in blood pressure, increased wall thickness and cross-sectional area of the aorta and decrease of elastin, collagen, actin content in rat model [7] and left ventricular fibrosis resulting in cardiac hypertrophy [15].

In recent years, polyphenols have attracted considerable attention as agents that protect cells or molecules from oxidative myocardial injury. Ellagic acid (EA) is one such polyphenolic phytonutrient [9] found to be an effective for antioxidant, anti-inflammatory, anti-hyperlipidaemic and anti-carcinogenic activities [6]. Moreover, it has been shown to be a potent benefit for chemopreventive, anti-fibrotic, anti-viral and anti-bacterial properties [5, 20, 21]. However, there is no evidence to support the preventive effects of ellagic acid on the structural remodeling of the heart in the hypertensive rat induced by nitric oxide deficiency. Therefore, the aim of this study was to investigate the preventive effect of ellagic acid on blood pressure, relative left ventricular weight and cardiac hypertrophy in the L-NAME-induced hypertensive rats.

Materials and methods

Animal and Experimental protocols

Male Sprague-Dawley rats weighing 200-240 g were obtained from the National Laboratory Animal Center, Mahidol

University, Salaya, Nakornpathom. All study animals were housed in stainless steel cages and maintained in an air-conditioned room (25.1±1oC) with 12:12 h light/dark cycle. They were fed with a standard chow diet (Chareon Pokapan Co. Ltd., Thailand) and tap water ad libitum at Northeast Laboratory Animal Center of the Faculty of Medicine, Khon Kaen University. All procedures and experimental protocols were reviewed approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 70/2555).

After one week of acclimatization, rats were randomly divided into 3 groups with 5 animals in each group: (1) control group receiving tap water, (2) hypertensive group received L-NAME (Sigma Chemical Co.) 40 mg/kg/day in their drinking water, and (3) L-NAME+EA (Sigma Chemical Co.) treated group received L-NAME 40 mg/kg/day with EA 15 mg/kg/day. The compound (EA) was dissolved in distilled water (DW) 1.5 mg/kg/day and administered orally for 4 weeks. The choice of EA dosage used in this study was selected on the basis of previous study in experimental model of animals which shown to be a potent benefit for biological properties of EA [9]. The body weight was measured every week until the end of experiment. After the end of experiment, the animals were sacrificed, their body weight (BW), weight of the heart (HW), left ventricular weight (LVW) were determined, and the LVW/BW ratio was calculated.

Blood pressure measurement

Systolic blood pressure (SBP) was measured non-invasively in conscious animals by using tail-cuff plethysmography (Blood pressure analyzer, model 29; IITC, Woodland Hills, California, USA) every week to assess the development of the L-NAME-induced hypertension during EA treatment. The mean values of three successive measurements were obtained from each rat.

Histomorphometric study of the heart

The hearts were rapidly removed and left ventricle was isolated and bisected coronally at the midventricular position, equidistant between base and apex. Then, the heart was 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 left ventricular wall thickness and cross section area were evaluated with Image-J NIH image analysis software as follows:

1. The left ventricular wall thickness was measured every 45 ° 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 was expressed as mean ± S.E.M. Statistical differences were evaluated by one-way analysis of variance (ANOVA) and followed by Student Newman-Keul's test to show specific group differences. All analysis was performed using Sigmastat software version 3.1 The p values<0.05 were considered statistically significant.

Results

Effect of EA on body weight

The body weight was observed every week during the experimental period. We found that the values of body weights were not significantly different among experimental groups (Figure 1).

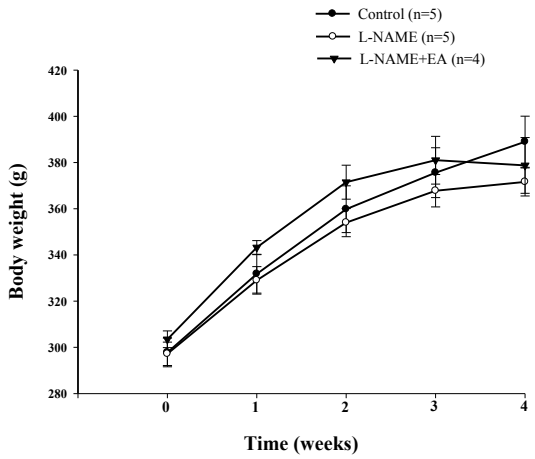

Figure 1 Effect of 4-week L-NAME and EA on body weight. Results are expressed mean±SEM

Effect of EA on systolic blood pressure

At the beginning of the experiment (week 0) SBP were not significantly different among the groups. Administration of L-NAME caused a significantly progressive increase in SBP during 4 weeks of its administration when compared with the control group ($p<0.05$). SBP in L-NAME-treated with EA group was significantly lower than those in L-NAME group since the first week until the end of the experiment ($p<0.05$). However, blood pressure of EA-treated group remained higher when compare to the control group (Figure 2).

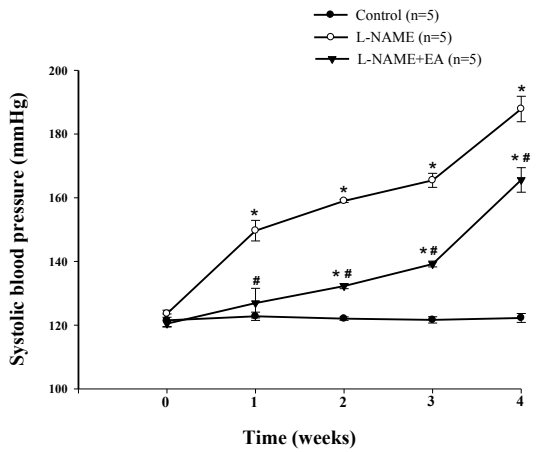

Figure 2 Effect of 4-week L-NAME and EA on systolic blood pressure. Results are expressed mean±SEM. *$p<0.05$ versus control group, #$p<0.05$ versus L-NAME group

Effect of EA on body weight and cardiac parameters

At the end of the experiment, body weight (BW), heart weight (HW), left ventricular weight (LVW) and LVW/BW ratio were not significantly different among groups (Table 1).

Effect of EA on wall thickness and cross sectional area of left ventricle

Administration of L-NAME only caused a significant increase in the left ventricular wall thickness when compare to the control group. The left ventricular wall thickness of L-NAME treated with EA was slightly smaller than those in L-NAME rats but not reach a statistically significant level. The cross-sectional area of left ventricle in L-NAME and L-NAME receiving EA were significantly greater than those in control group, but there were no significant difference in cross-sectional area between these two (Table 2).

Discussion

This study examined the preventive effect of EA on blood pressure and cardiac wall changes in L-NAME 4-week induced hypertension. We found that the animal received L-NAME had an increased of SBP since the first week until the end of the experiment. This finding is consistent with the other observations [10, 13, 15, 16].

Table 1 Effect of EA on general biological parameters of heart

	BW (g)	HW (g)	LVW (g)	LVW/BW (mg/g)
Control	386.80±11.60	1.37±0.04	0.97±0.02	2.51±0.08
L-NAME	377.25±4.49	1.43±0.05	1.02±0.04	2.71±0.13
L-NAME+EA	381.00±13.61	1.42±0.04	1.04±0.05	2.74±0.16

Values are mean±SEM. Each group contains four to five animals.

Table 2 Effect of EA on wall thickness and cross sectional area of left ventricle

	Wall thickness (mm)	Cross sectional area (mm^2)
Control	2.18±0.08	47.70±1.37
L-NAME	2.58±0.05*	56.11±0.88*
L-NAME+EA	2.39±0.09	55.87±3.01*

Values are mean±SEM. Each group contains four to five animals. *p<0.05 versus control group

It has been known that blood pressure is determined by total peripheral resistance and cardiac output, an increase in one of these factors can cause hypertension. It was well known that NO is a major endothelium derived relaxing factor that induces relaxation of vascular smooth muscle cells [19]. Thus, changes in arterial blood pressure or total peripheral resistance after acute L-NAME administration are connected with keeping balance between vasoconstriction and vasodilatation [1]. In addition, we examined the thickness of the left ventricular wall. Our present study revealed that the left ventricular wall thickness and the cross-sectional area of 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. In NO deficiency condition, the increased accumulation of superoxide generation occurred and led to an inflammation, vascular cell growth and increased collagen deposition in both heart and vessel [11, 18, 22]. Regarding the relative heart weight, there was no significant difference among groups. However, the relative heart weight of L-NAME and L-NAME treated with EA were slightly higher than control. This might be the effect of animal number since this is the pilot study.

In this study, we also investigated the preventive effect of EA on blood pressure, cardiac dimension and weight. We found that administration of EA 15 mg/kg/day for 4 weeks inhibited progression of SBP. There is accumulating evidence that a phenolic compound provides an anti-hypertensive property [10, 13, 23]. The mechanism involved in the decrease of blood pressure produced by EA is unknown. It could be hypothesized that the decrease in blood pressure might be an antioxidant property of EA because several lines of evidence have shown that antioxidant substances significantly decrease superoxide generation [10, 13, 23].

Interestingly, although administration of EA 15 mg/kg/day significantly inhibited the development of hypertension, it did not have a significantly reduced left ventricular dimension. However, the left ventricular wall thickness of L-NAME treated with EA was slightly smaller than L-NAME rats. It was likely that EA trended to reduce wall thickness when compare to L-NAME group. It has been reported that dosage of the substance was influence to the response of various organs [10]. Another, hypothesis is that the significant difference might be found if animal number is increased.

Conclusion

In conclusion, inhibition of NO synthesis by L-NAME administration promotes an increase of blood pressure, without effect on alterations of general biological parameters of the heart but it increases wall thickness and cross sectional area of left ventricle. EA showed to be a potent benefit for decrease blood pressure

and trended to reduce wall thickness. This could be its antioxidant property.

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