Asiatic Acid Improves Endothelium-Dependent Vasorelaxation in Conduit and Resistance Vessels Isolated from Metabolic Syndrome Rats

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Background and Objective: In previous study, we have reported an antihypertensive effect of asiatic acid (AA), a triterpinoid compound, in high carbohydrate-high fat diets (HCHF)-induced metabolic syndrome (MS) rats. This study aimed to evaluate the effects of AA on vascular function in thoracic aorta and mesenteric vascular beds isolated from MS rats.

Method: Rats were fed with HCHF diets plus 15% fructose in drinking water for 12 weeks to induce MS and orally administered with vehicle or AA (20 mg/kg BW/day) for the last three weeks of the study. At the end of study, systolic blood pressure (SP), heart rate (HR) and metabolic indices were measured. Thoracic aorta and mesenteric vascular beds were isolated and set up for vascular function study. Vascular responses to acetylcholine (Ach) and sodium nitroprusside (SNP) were examined.

Results: Rat fed with HCHF diet exhibited signs of MS including, high SP, dyslipidemia and hyperinsulinemia. AA restored all metabolic abnormalities and SP in MS rats ($p<0.05$). Additionally, vascular responses to Ach were significantly blunted in both thoracic aorta and mesenteric vascular beds of MS rats comparing to the response in control rats ($p<0.05$). The improvement of vascular responses to Ach was observed in MS rats treated with AA ($p<0.05$), while the relaxation response to SNP did not differ in all preparations, indicating the normal vascular smooth muscle cells function.

Conclusion: This study suggests that AA alleviates hypertension in MS rats. This might be related to improving endothelium-dependent vasorelaxation in both thoracic aorta and mesenteric vascular beds isolated from MS rats.

Key words: Asiatic acid, Metabolic syndrome, Vascular function

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Proceeding

Introduction

Metabolic syndrome (MS) is a group of metabolic abnormalities including, central obesity, insulin resistance, impaired glucose tolerance, hypertension and dyslipidemia which lead to increase risk of developing cardiovascular diseases and diabetes mellitus.[1-3] Furthermore, it is noteworthy that endothelial dysfunction was clearly observed in MS rats.[4,5]

Asiatic acid (AA) is a triterpenoid compound isolated from plant *Centella asitica*. It has been shown to have biological acidities including antioxidant, antihyperlipidemic and anti-diabetic effects.[6-8] In previous studies, we reported antihypertensive and anti-inflammatory effects of AA in high carbohydrate-high fat diet (HCHF)-induced MS rats.[9,10] However, the effects of AA on vascular function in HCHF induced MS rats

have not been studied. We hypothesized that AA treatment could improve vascular function in MS rats.

The present study is aimed to evaluate the effect of AA on vascular function in thoracic aorta and mesenteric vascular beds isolated from HCHF induced metabolic syndrome rats.

Methods

Chemicals

AA was obtained from Sigma-Aldrich (St. Louis, MO, USA) (purity >95%).

Animals

Male Sprague-Dawley rats (220-240 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 the 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 36/2555).

Experimental designs

After one week of acclimatization, the animals were randomly divided into two main groups. Group 1, the normal control group (C); received a standard chow diet (Chareon Pokapan Co. Ltd., Thailand) and drinking tap water, Group 2, MS group (MS); received HCHF diet together with 15% fructose in drinking water for 12 weeks to induce metabolic syndrome. After the induction period, MS rats were divided again in to MS group and MS plus AA group (MS+AA) which received AA at a dose of 20 mg/kg BW per day for another three weeks.

Assessment of metabolic and biochemical parameters

At the end of the study, SP and HR were measured using tail-cuff plethysmography (IITC model 179 blood pressure analyser) method. Insulin level was measured using a Rat Insulin ELISA Kits (Millipore Corporation, Billerica, MA, USA). Lipid profiles were analyzed at Clinical Chemistry Laboratory Unit of the Faculty of Associated Medical Sciences, Khon Kaen University, Thailand.

Vascular function study

At the end of experiment, the animals were killed by overdose of sodium pentobarbital followed by exsanguinations. Mesenteric vascular beds were collected and perfused with physiological Krebs' solution. Vascular responses to vasoactive agents, acetylcholine (Ach, 1nM-0.01μM) and sodium nitroprusside (SNP, 1nM-0.01μM) were assessed under methoxamine-rised tone conditions. Moreover, the thoracic aorta was rapidly removed and cut into rings of 2-3mm width for tension measurement. They were mounted in 15 ml bath containing Krebs' solution at 37°C and gassed with air. Isometric contractions were recorded with a resting tension of 1g using a transducer connected to a 4-channel bridge amplifier and a PowerLab A/D converter and a PC running Chart v5 (A.D. Instrument, Australia). Acetylcholine (Ach-0.01μM-3μM final concentrations) induced endothelial mediated-relaxations and sodium nitroprusside (SNP) were assessed by pre-contracting with phenylephrine (10μM) and relaxation expressed as % of the phenylephrine-induced contraction.

Statistical analysis

Data were expressed as mean ± S.E.M. The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Turkey's tests. A *p*-value of less than 0.05 was considered a statistical significance.

Results

Metabolic parameters

Rats fed with HCHF diet showed a significant increased in SP, HR and insulin level comparing to those of control group ($p<0.05$) (Table 1). Moreover, MS rat exhibited abnormal lipid profiles including increased of total cholesterol and triglycerides levels while decreased HDL-C level when compared with those of control rats ($p<0.05$) (Table 1). Interestingly, treatment with AA (20 mg/kg BW) for 3 weeks significantly reduced SP, HR, insulin level (Table 1) and also improved total cholesterol and triglycerides levels, HDL-C level comparing to those of MS rats ($p<0.05$) (Table 1).

Vascular responses to vasoactive agents

Thoracic aorta assessment

Ach (0.01M-3 μM) caused an endothelium-depen-

Table 1 Effects of Asiatic acid (AA) on SP, HR and metabolic parameters in all expermental groups (n = 5-6/ group).

Parameters	Normal control	MS	MS+AA
SP (mmHg)	119.3 ± 2.2	154.3 ± 1.1*	137.2 ±1.3* #
HR (beats/min)	353.1 ± 17.7	426.0 ± 9.0*	396.8 ± 5.9* #
Insulin level (ng/ml)	0.5 ± 0.1	3.8 ± 0.5*	1.6 ± 0.3* #
Cholesterol level (mg/dL)	51.8 ± 2.3	81.2 ± 2.3*	63.6 ± 3.2* #
Triglyceride level (mg/dL)	25.8 ± 3.0	71.5 ± 2.7*	26.2 ± 2.7 #
HDL-C level (mg/dL)	40.2 ± 1.2	14.5 ± 1.5*	34.6 ± 0.6* #

*p<0.05 vs normal control, #p<0.05 vs MS

dent relaxation in concentration-dependent manner in all preparations (Fig. 1A). Vasorelaxation responses to Ach were significantly blunted in MS preparations comparing to the response in control rats (Ach (1 μM), 39.5 ± 5.1 vs. 16.3 ± 1.6 % contraction) (p<0.05). AA Supplementation improved the response in MS rats (Ach (1 μM), 21.6 ± 3.1 vs. 39.5 ± 5.1 %contraction) (p<0.05). However, there was no significant difference in vascular responses to SNP in all groups of rats (Fig. 1B).

Mesenteric vascular bed assessment

Similarly to the large arteries, Ach (0.1μM-0.1mM) caused an endothelium-dependent relaxation in concentration-dependent manner of all preparations (Fig. 2A). Vascular responses to Ach were significantly impaired in MS preparations comparing to the response in control rats (Ach,10 μM), 12.9 ± 4.1 vs. 42.9 ± 4.4 mmHg) (p<0.001). Treatment with AA showed an improvement in responses to Ach (Ach,10 μM), 20.8 ± 4.5 vs. 12.9 ± 4.1 mmHg) comparing to the response in MS rats (p<0.05). However, the relaxation responses to SNP did not differ in all groups indicating the normal vascular smooth muscle cells function (Fig. 2B).

Conclusion

This present findings suggest that HCHF diet caused signs of MS in rats such as high blood pressure, insulin resistance, high cholesterol and low HDL-C. AA improved insulin sensitivity, indicating by a reduction of serum insulin level in MS rats. Dyslipidemia were also attenuated with AA treatment. These findings were consistent with the study in HCHF diet-induced MS rats.[8, 9, 11] Furthermore, MS rats exhibited high blood pressure with decreased response to Ach. On the other hand, the response to SNP, a nitric oxide donor did not alter in both preparations. These results indicated that endothelial dysfunction was occurred in MS rats. It is well established that vascular dysfunction is a major cause of atherosclerosis and high blood pressure in MS.[12] This study supported the possible mechanism of AA in alleviation of hypertension. That effect could be via improving the endothelial function in both conduit and resistance vessels.

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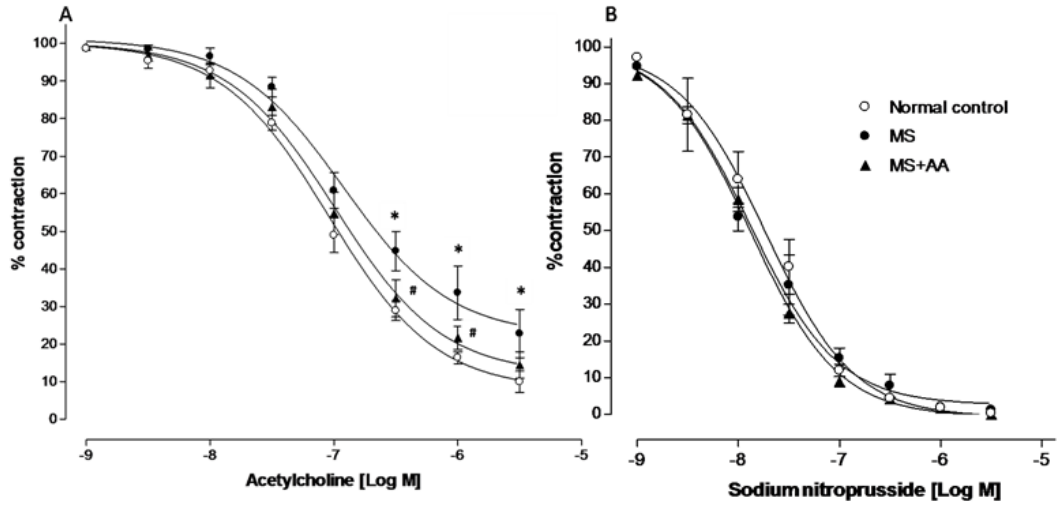

Figure 1 Effect of asiatic acid on vascular responses to Ach (A) and SNP (B) in rat thoracic aorta in all experimental groups. Data are presented as mean ± S.E.M. (n = 5-6/group). * p<0.05 vs. control, # p<0.05 vs. MS.

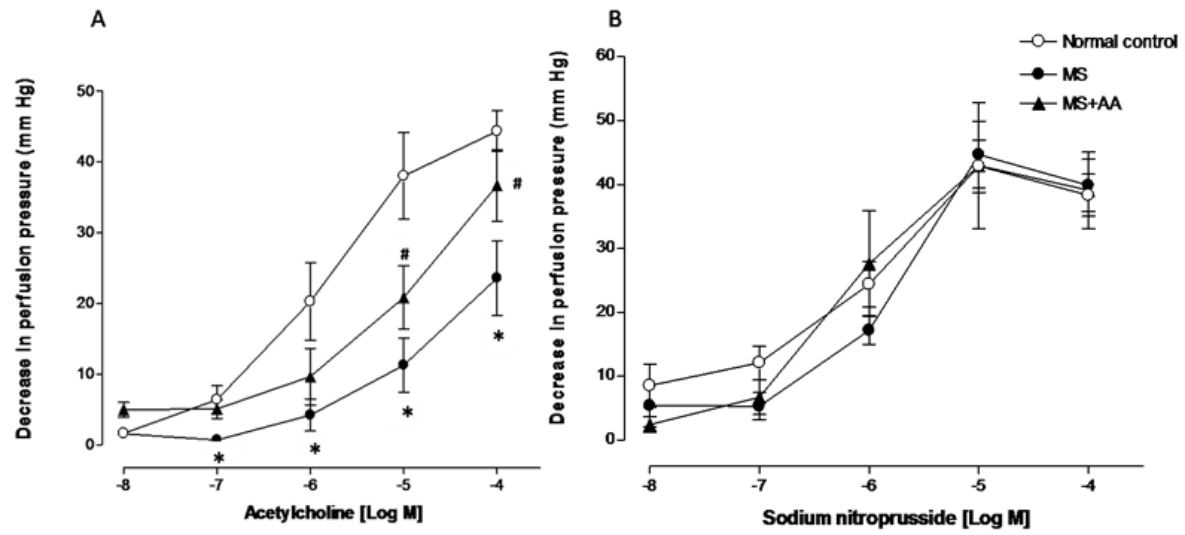

Figure 2 Effect of asiatic acid on vascular responses to Ach (A) and SNP (B) in mesenteric vascular beds in all experimental groups. Data are presented as mean ± S.E.M. (n = 5-6/group). * p<0.05 vs. control, # p<0.05 vs. MS.

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