

Vasopurus의 LDL r^{-/-} Mice에서 항동맥경화 효능

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Protective Effect of *Vasopurus* on Atherosclerosis Induced by Dietary Cholesterol in LDL r^{-/-} Mice

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ABSTRACT

Objectives : The protective effect of *Vasopurus*, a mixture of three medicinal plants, on atherosclerosis induced by dietary cholesterol in low density lipid (LDL) receptor deficient mice was studied.

Methods : Experimental groups were divided into three groups of six animals each; a normal group (N); a high cholesterol group (HC), a high cholesterol plus *Vasopurus* group (HCVA). The experimental groups were fed for 6 months.

Results : *Vasopurus* supplementation significantly lowered plasma total cholesterol and LDL cholesterol concentrations compared with the high cholesterol (HC) diet group. In addition, supplementation with *Vasopurus* significantly increased fecal cholesterol contents compared to mice fed a HC diet. Mice whose diet was supplemented by *Vasopurus* showed considerably fewer atherosclerotic plaques in the aortic valves of heart and aortas compared to mice receiving the HC diet.

Conclusions : These results indicate that *Vasopurus* has efficacy in anti atherosclerosis medication.

Key words : *Vasopurus*, LDL receptor deficient mice, anti atherosclerosis, atherosclerotic plaques

Introduction

Atherosclerosis is the major cause of morbidity and mortality in western and eastern societies^{1,2}. Abundant evidence demonstrates that elevated plasma triglyceride, total cholesterol and low density lipoprotein (LDL) cholesterol (LDL C), together with decreased high density lipoprotein (HDL) cholesterol (HDL C) are

primary risk factors that are positively associated with the development and progression of atherosclerosis³⁻⁵. A strategy to prevent or to treat atherosclerosis and reduce the incidence of cardiovascular disease events is to target hyperlipidemia by drugs and/or dietary intervention⁶. Drug therapy, especially the use of statins, is highly beneficial to hypercholesterolemic populations through the lowered blood cholesterol and, to a lesser

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erent, lowered triglycerides levels^{7,8)}. However, severe side effects are associated with the use of the statin class of lipid lowering medications⁹⁻¹¹⁾.

On the other hand, the cholesterol lowering effect of dietary plants has been well studied and various plants are helpful in lowering plasma cholesterol levels and encouraging safety profile^{12,13)}. Dietary plants are considered to be a useful means to prevent disorders such as atherosclerosis.

Vasopurus is a mixed powder of three medicinal plants (*Atractylodes Rhizoma*, *Thujae orientalis Folium* and *Glycyrrhizae Radix*), which prevented atherosclerosis in rabbits¹⁴⁾. However, the anti atherogenic effects of *Vasopurus* have not yet investigated in a mouse model of atherosclerosis. Presently, we studied whether the dietary administration of *Vasopurus* could affect the cholesterol profiles and retard atherosclerosis in LDL receptor deficient (LDLr^{-/-}) mice.

Materials and Methods

1. Sample Preparation

Vasopurus was provided by Hyolim Bio Co. Ltd. (Korea) as a powdered mixture of the three aforementioned plants in 6:2:1 (w/w/w) proportions.

2. Animals and diets

LDL r^{-/-}, B6.129S7 Ldlr^{tm1Her}/J mice were obtained from Jackson Laboratory (Bar Harbor, Maine, USA). Animals were kept in a 21±2°C room maintained under 55±5% humidity and a 12 h light dark cycle and acclimatized to the facility for 7 days before or 7 days

of 7 experiments. Nine week old mice were randomly divided into three groups of six animals each. The treatment groups included untreated normal (N) group, high cholesterol (HC) group who were fed a dayndard commercial rat chow (Sam Yng Co., Korea) supplemented the 1.25% of cholesterol, and high cholesterol plus *Vasopurus* (HCVA) group who received 7 dame HC diet the 1.25% *Vasopurus*. All animals were fed for 6 months, and the composition of the experimental diet is shown in Table 1. The mice were fed *ad libitum* the facidiet and were permitted free access to tap water. The weight was recorded once weekly, whereas diet and water intake were recorded every three days throughout the study.

3. Plasma and tissue preparations

At the end of the 6 months treatment period after an overnight fast, mice were sacrificed under ether anesthesia, and blood was collected in a heparinized tube. The collected blood was centrifuged at 3,000 rpm for 20 min, and the serum was stored at 80°C until processed. After blood collection, the liver and kidney were removed immediately, washed in ice cold saline, and weighed after blotting on a filter paper. The whole liver was cut into three portions and quickly freeze clamped in liquid nitrogen, and stored at 80°C until analysis.

4. Biochemical analysis

Plasma total cholesterol (TC), triglyceride (TG), LDL C and HDL C were determined enzymatically using commercially available kits (Asan Pharm, Korea). Fecal lipids were extracted as previously described¹⁵⁾. Briefly, about 0.5 g of feces were extracted in 10 mL of Folch solution (chloroform:methanol, 2:1, v/v). After extraction, the lipid solution was filled to 10 mL with Folch solution and then fecal cholesterol and TG contents were measured with a commercial kit (Asan Pharm).

5. Histological examinations

After mice were euthanatized, the right atrium was removed and hearts and aortas were perfused with phosphate buffered saline (PBS) through the left ventricle. Hearts were embedded in OCT and frozen on dry ice. Aortas were fixed overnight with 4% paraformaldehyde in PBS, dissected from the proximal

Table 1. Composition of Experimental Diets

Ingredient (g)	N	HC	HCVA
Casein	20.00	20.00	20.00
DL Methionine	0.30	0.30	0.30
Corn starch	15.00	15.00	15.00
Sucrose	38.55	38.55	38.55
Cellulose	5.00	5.00	5.00
Cholesterol		1.25	1.25
Mineral mixture	3.50	3.50	3.50
Vitamin mixture	1.00	1.00	1.00
Choline bitartrate	0.20	0.20	0.20
Sodium cholate	0.20	0.20	0.20
<i>Vasopurus</i> powder*			1.25

* : *Vasopurus* powder : Mixture of *Atractylodes ovata* (Thunb.) DC, *Thuja orientalis* L. and *Glycyrrhiza glabra* L. N : normal diet. HC : high cholesterol. HCVA : high cholesterol + 1.25% *Vasopurus*.

ascending aorta to the bifurcation of the iliac artery, and had adventitia removed. Aortas were snap-frozen in OCT compound. Frozen sections (6 μm) of mouse aortic arches and hearts were prepared. Sections of aortas and hearts were stained with hematoxylin eosin (H&E) and digitally photographed at a fixed magnification (x400).

6. Statistical analysis

The results were expressed as mean ± SD and statistically analyzed by analysis of variance (ANOVA). Duncan's multiple range test was performed to determine significant differences among the groups and differences at p<0.05 were considered to be significant.

Results

1. Changes in Body and organ weights

Atherosclerosis was induced experimentally by feeding LDLr^{-/-} mice a cholesterol rich diet for 6 months. No significant differences were observed in the body weights between the three groups (Table 2). The liver weight in HCVA group was significantly reduced in comparison with the HC group, which was similar to the N group (Table 2), but the weight of the kidney was not different among the groups.

Table 2. Effects of *Vasopurus* on Body Weights Gain, Liver and Kidney Weights in LDLr^{-/-} Mice Fed a High Cholesterol Diet

Group	N	HC	HCVA
Initial body weight (g)	22.44±0.61	22.78±0.59	22.76±1.31
Final body weight (g)	30.02±2.30	30.13±1.07	29.36±1.37
Weight gain (g)	7.58±1.53	7.35±0.94	6.66±1.38
Liver (g)	4.46±0.12 ^b	4.91±0.13 ^a	4.60±0.16 ^b
Kidney (g)	1.13±0.09	1.09±0.02	0.99±0.06

The values are mean ± S.D. (n=6). Values with different superscripts are significantly different by ANOVA with Duncan's multiple comparison test at p<0.05. N : normal diet. HC : high cholesterol. HCVA : high cholesterol + 1.25% *Vasopurus*.

2. Effects of *Vasopurus* on plasma and fecal lipid levels

Plasma levels of TC were significantly higher in the HC diet group, compared with the N group (Table 3). In the HCVA group the TC levels were significantly reduced as compared with the HC diet group. LDL C and HDL C levels were significantly decreased in the

HCVA group as compared to the HC diet group. In addition, supplementation of the HC diet with *Vasopurus* produced a slight increase in fecal TG levels compared to the HC group (Table 3), but fecal cholesterol levels were significantly reduced in comparison with the HC group.

Table 3. Effects of *Vasopurus* on Cholesterol Levels of Plasma and Feces in LDLr^{-/-} Mice Fed a High Cholesterol Diet

Group	N	HC	HCVA (mg/dL)
Plasma			
Total cholesterol	302.50±24.42 ^c	995.22±37.36 ^a	707.59±28.95 ^b
LDL cholesterol	116.50±15.73 ^c	646.76±23.89 ^a	408.30±17.51 ^b
HDL cholesterol	127.50±9.99 ^f	282.60±22.58 ^a	236.00±16.23 ^b
Feces			
Triglyceride	349.40±14.14 ^d	310.61±18.43 ^b	318.49±12.71 ^b
Total cholesterol	171.72±11.81 ^c	236.32±12.52 ^b	263.99±10.26 ^a

The values are mean ± S.D. (n=6). Values with different superscripts are significantly different by ANOVA with Duncan's multiple comparison test at p<0.05. N : normal diet. HC : high cholesterol. HCVA : high cholesterol + 1.25% *Vasopurus*.

3. Histological analysis

Examination of aortic roots in hearts stained with H&E for lipids showed decreased atherosclerotic plaque formation in the HCVA group compared with the HC group (Fig. 1). We then analyzed the difference of the atherosclerotic plaques in aortic arch sections of LDLr^{-/-} mice. The HCVA group also showed thick aortic valves with unequal ridges but the degree of lesions was scant compared to that of the HC group (Fig 2).

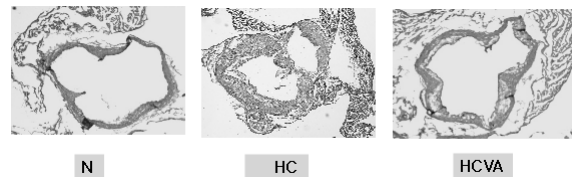


Fig. 1. Effects of *Vasopurus* on atherosclerotic lesions in LDLr^{-/-} mice fed a high cholesterol for 6 months

Representative H&E stained aortic sinus from LDLr^{-/-} mice (×400). Symbols. N : control group fed a normal diet. HC : high cholesterol fed diet group. HCVA : high cholesterol + 1.25% *Vasopurus*.

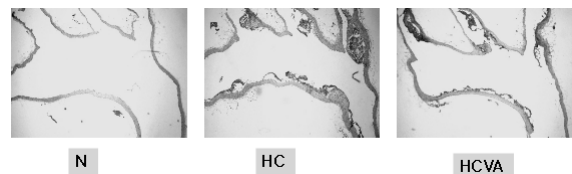


Fig. 2. Effects of *Vasopurus* on atherosclerotic lesions in LDLr^{-/-} mice fed a high cholesterol for 6 months

Representative H&E stained aortas from LDLr^{-/-} mice (×400). Symbols. N : normal diet. HC : high cholesterol. HCVA : high cholesterol + 1.25% *Vasopurus*.

Discussion

This study verified a preventive effect of *Vasopurus* on atherosclerosis induced by feeding HC diet to LDLr^{-/-} mice for 6 months. In the present study, LDLr^{-/-} mice were fed a HC diet supplemented with 1.25% *Vasopurus* for 6 months. Dietary supplementation with *Vasopurus* significantly reduced liver weight by HC diet but did not affect body weights during the experimental period. In addition, plasma cholesterol concentrations in the HCVA diet group decreased in comparison with the HC diet group. The reduced plasma total cholesterol was associated with a decrease of its LDL fraction, which is a major and potentially modifiable risk factor of cardiovascular diseases and a target of many hypocholesterolemic therapies. This finding suggests that the cholesterol lowering activity of *Vasopurus* appears to be due to the enhancement of LDL cholesterol catabolism through hepatic receptors, consistent with previous observations¹⁶⁾.

Medicinal plants exhibit a wide array of pharmacological functions that include hypolipidemic, antioxidative, anti proliferative, immunomodulatory and anti inflammatory activities¹⁷⁾. Previous findings revealed that *Vasopurus* potently protects hyperlipidemic rabbits against atherosclerosis¹⁴⁾. One of the plants that comprise *Vasopurus* is *Atractylodes ovata* (Thunb.) DC, which contains sesquiterpen alcohol and isoprenoidal substances. This is interesting, given that the intermediates of cholesterol biosynthesis such as farnesyl pyrophosphate and mevalonic acid also have an isoprenoidal structure. This implies that *Atractylodes ovata* may also have an antihypercholesteremic effect by virtue of the competitive inhibition of its constituents with farnesyl pyrophosphate and mevalonic acid¹⁴⁾. Therefore, *Vasopurus* might constitute a good candidate for the treatment of atherosclerosis by lowering plasma cholesterol levels.

Supplementation of *Vasopurus* increased fecal cholesterol at a significant level compared with esterol significant cholesterol lowering effect of *Vasopurus* might be due to an increased excretion of cholesterol through fecal sterol excretion.

Atherosclerosis occurs on inner walls of blood vessels regardless of the size of arteries, and is especially found near the bifurcation of blood vessels^{2,3,18)}. It is considered to be a hemodynamic problem. Its major symptoms are motor abnormality and cardiac dysfunction following ischemia in a heart and a brain⁴⁾. One of key factors provoking atherosclerosis in experimental animals (rabbits and LDLr^{-/-} mice) and humans is increased blood cholesterol (hypercholesterolemia). Over 90% of

cholesterol in the body consists of a cholesterol ester formed by binding with fatty acid³⁾. We analyzed atherosclerotic lesion size in the heart and aorta in each dietary group of LDLr^{-/-} mice. The HC group showed advanced atherosclerotic lesions in the aortic root and sinus. In contrast, supplementation of HC diet with *Vasopurus* marked reduced aortic lesion size.

Conclusion

Vasopurus supplementation significantly lowers plasma total cholesterol and LDL cholesterol concentration compared with HC diet group. In addition, *Vasopurus* dietary supplementation significantly increases fecal cholesterol excretion and minimizes formation of atherosclerotic plaques in the aortic valves of heart and aortas compared with that of the HC group. Therefore, *Vasopurus* is capable of decreasing blood cholesterol level and prevalence of atherosclerosis. Although the precise mechanism of the active substances in *Vasopurus* remains to be discovered, this medicinal plant might offer a novel approach for the prevention of atherosclerosis.

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