Original Article

Anti-atherosclerosis action of the traditional Korean medicine Bok Ryung Ban Ha -Tang in ApoE KO mice

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Received March 15, 2016; Accepted September 7, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Atherosclerosis is characterized by vascular obstruction from the deposits of plaque, resulting in reduced blood flow. Here, we investigate that dynamic changes of bodyweight, liver weight, lipid levels, atherosclerotic lesion and liver steatosis, during the progression of atherosclerosis in ApoE KO mice. Male ApoE KO mice were fed a high fat diet (western type diet, WD) for 16 weeks. BRBHT; Bok Ryung Ban Ha -Tang, treatment of WD-fed atherosclerosis mice significantly reduced body weight, liver weight, blood pressure and lipid levels relative to the WD-fed mice. Lipid levels of T-chole, LDL, TG, Glucose were significantly decreased in the BRBHT treat mice compared with the WD-fed mice, also AST, ALT were significantly changed in the BRBHT mice compared with the levels in the WD mice. Histological examination showed that the atherosclerotic lesions were reduced in the WD with BRBHT group compared with the WD group. Furthermore, the administration of BRBHT decreased fat accumulation in hepatic. Taken together, the present data indicate that BRBHT prevents atherosclerosis in WD-induced ApoE KO mice by regulation of lipid and liver steatosis.

Keywords: Atherosclerosis, apolipoprotein E-deficient mice, Bok Ryung Ban Ha -Tang, liver steatosis, cholesterol

Introduction

Atherosclerosis is multifactorial disease of the large arteries and the leading cause of morbidity and mortality in industrialized countries [1, 2]. Early atherosclerotic lesions are characterized by the accumulation of fatty streaks consisting of abnormal lipids, lipid-laden foam cells in the vessel wall intima. With disease progression, more complicated inflammatory processes are involved and atherosclerotic plaques are covered with fibrous caps form in the aortic vessel walls [3]. Furthermore, High blood pressure is a major cause of atherosclerosis, the artery-clogging process that leads to heart attacks and strokes [4].

Numerous pharmacological approaches for the prevention and treatment of atherosclerosis have been suggested. Treatment for atherosclerosis may include lifestyle changes, medical surgery and the administration of drugs that prevent blood clots by lower cholesterol or blood pressure [5]. Clinically available anti-atherosclerosis agents include Statin (HMG-CoA reductase inhibitors), which is a centrally acting in the production of cholesterol in the liver [6]. However, these agents have been reported to cause undesirable side-effects, including pain, tiredness, liver and muscle problems with ocular (such as, blurred vision) side effects [7]. Therefore, due to the limited usage of these pharmacological agents, there is a demand for alternative therapies, including the use of herbal products, that have minimal side effects [8]. For example, Doinseungi-tang, a traditional herbal medicine composed of 5 species, has been reported to be an effective treatment for atherosclerosis. In particular, Doinseungo-tang reduced atherosclerotic lesion and lipid levels [9].

Bok Ryung Ban Ha -Tang, which is one of the herbal mixtures. In prescription is three species composed of Pinellia ternata (Thunb.) Breit, Wolfiporia extensa, Zingiber officinale, that have various pharmacological on the traditional medicinal prescription has been used orally for...
promoting blood circulation to remove blood stasis and anti-inflammation [10].

Traditional Korean herbal medicine has been widely used in the treatment of atherosclerosis-related disorders [11]. However, the anti-atherosclerosis effects of BRBHT have not previously been studied. In this study, we investigated the effects of BRBHT on bodyweight, serum lipid levels, atherosclerotic lesion and liver steatosis WD-induced atherosclerosis.

Materials and methods

Preparation of BRBHT

The herbs were purchased from Naemome-Dah Medicinal Herbs Co. (Ulsan, Korea) and authenticated based on their microscopic and macroscopic characteristics by the classification and identification committee of the Korea Institute of Oriental Medicine (KIOM).

The formula of BRBHT consists of three herbs, including *Pinellia ternata* (Thunb.) (120 g), *Poria cocos* (80 g), *Zingiber officinale* Rosc. (80 g), which were mixed and ground into a crude powder. The total mixture (280 g) was boiled in distilled water (1:10, v/v) at 100°C for 2 h and the extract was filtered, lyophilized and subsequently stored at -20°C. The yield of BRBHT aqueous extract was 11.25% (w/w) (Table 1).

**Table 1. Composition of Bok Ryung Ban Ha -Tang**

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Herbal name</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pinellia ternata</em> (Thunb.) Breit</td>
<td><em>Pinellia Tuber</em></td>
<td>120</td>
</tr>
<tr>
<td><em>Wolfiporia extensa</em></td>
<td><em>Poria cocos</em></td>
<td>80</td>
</tr>
<tr>
<td><em>Zingiber officinale</em></td>
<td><em>Ginger</em></td>
<td>80</td>
</tr>
</tbody>
</table>

Body weight and blood pressure

Body weight were measured once a week and Blood pressure was monitored using a noninvasive tail-cuff CODA™ system (Kent Scientific; Torrington, CT, USA) as previously described [12].

Liver weight

Subsequent to blood collection, the livers were removed from the mice and immediately weighted.

Analysis of serum markers

Blood was collected from the aorta under light anesthesia and stored on ice for 30 min before centrifugation at 13,000 rpm at 4°C for 10 min, after which they were stored at -80°C. Serum levels of T-chole, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglyceride (TG), glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine were measured with an automatic analyzer (Hitachi Co., Tokyo, Japan).

Atherosclerotic lesion

Mice were anesthetized and euthanized after 16 weeks of WD or Con. The aortas were washed with phosphate-buffered saline (PBS) and then fixed with 10% paraformaldehyde overnight. The adventitia was cleaned thoroughly under a dissecting microscope, and fixed aortas were stained with Oil Red O stock solution (0.3% w/v in isopropyl alcohol) for 1 h.
Histopathology

Mice were deeply anesthetized with sodium thiopental and subsequently perfused with cold PBS, followed by fixation with 10% (w/v) paraformaldehyde overnight. Livers were embedded in paraffin and sectioned serially at 4 μm. The sections were stained with H&E. The stained sections were analyzed by light microscopy (Olympus optical Co., Tokyo, Japan).

Data analysis

Values are expressed as the mean ± standard error of the mean (SEM). Statistical comparisons were performed using unpaired Student’s t-tests and analysis of variance (ANOVA) for repeated measures followed by post hoc pairwise comparisons in SigmaPlot software version 13.0 (Systat Software Inc.; San Jose, CA, USA). Differences were considered significant at P < 0.05.

Results

Effect of BRBHT on body weight and liver weight in ApoE KO mice

To investigate BRBHT possesses anti-atherosclerosis activities mice were fed with normal diet (Control; Con), WD or WD supplemented with BRBHT for 16 weeks, and their body weight monitored. After 3 weeks feeding, the WD group showed significantly increased body weight compared with that of the control (P < 0.001). BRBHT supplementation ameliorated the increased body weight of WD fed mice (P < 0.001; Figure 1A). As shown in Figure 1B, the body weight gain of the WD group was also increased compared to the control (P < 0.001), and the BRBHT supplementation attenuated this increased (P < 0.001). These results revealed that the supplementation of BRBHT...
Anti-atherosclerotic effects of Bok Ryung Ban Ha-Tang

Table 3. Effect of BRBHT on Blood pressure in ApoE KO mice fed a WD

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>WD</th>
<th>BRBHT 150 mg/kg</th>
<th>Atorvastatin 10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean pressure</td>
<td>93.0±1.30</td>
<td>117.0±5.48**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diastolic</td>
<td>108.0±2.84</td>
<td>140.0±7.04**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Systolic</td>
<td>78.0±3.27</td>
<td>94.0±4.50*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>90.0±3.20*</td>
<td>98.0±3.25*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>107.0±3.59**</td>
<td>119.0±5.40*</td>
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<td></td>
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<td></td>
<td>74.0±3.00**</td>
<td>77.0±2.16**</td>
</tr>
</tbody>
</table>

Metabolic characteristics were measured from ApoE KO mice fed a WD for 16 weeks. Abbreviations: ApoE KO, ApoE Knockout; WD, western diet; BRBHT, Bok Ryung Ban Ha-Tang. Values are expressed as the mean ± SEM. n=8; ***P < 0.001, vs. ApoE KO mice without WD (Con; Normal diet). **P < 0.01, vs. ApoE KO mice without WD (Con; Normal diet).

Table 4. Effect of BRBHT on Biochemical parameters in serum of ApoE KO mice fed a WD

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>WD</th>
<th>BRBHT 150 mg/kg</th>
<th>Atorvastatin 10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T-chole (mg/dL)</td>
<td>479.0±28.32</td>
<td>1232.57±72.45***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDL (mg/dL)</td>
<td>75.29±3.11</td>
<td>328.71±35.22***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDL (mg/dL)</td>
<td>19.43±2.14</td>
<td>12.14±1.64*</td>
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<tr>
<td></td>
<td></td>
<td>TG (mg/dL)</td>
<td>73.0±9.58</td>
<td>181.43±80.05</td>
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<tr>
<td></td>
<td></td>
<td>Glucose (mg/dL)</td>
<td>202.29±18.73</td>
<td>376.57±72.57*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AST (U/L)</td>
<td>135.57±32.26</td>
<td>499.01±48.63***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALT (U/L)</td>
<td>72.00±19.37</td>
<td>413.43±75.48***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Creatinine (mg/dL)</td>
<td>0.22±0.01</td>
<td>0.32±0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>929.71±33.74*</td>
<td>965.67±31.90*</td>
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<td></td>
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<td></td>
<td>250.57±15.05*</td>
<td>271.17±16.22</td>
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<td></td>
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<td></td>
<td>16.29±1.52</td>
<td>15.00±1.54</td>
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<td></td>
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<td></td>
<td>44.14±5.27*</td>
<td>89.83±12.99</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>271.43±20.35*</td>
<td>240.50±37.77*</td>
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<td></td>
<td></td>
<td></td>
<td>224.57±29.69*</td>
<td>313.33±76.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.23±0.03</td>
<td>0.23±0.03</td>
</tr>
</tbody>
</table>

Metabolic characteristics were measured from ApoE KO mice fed a WD for 16 weeks. Abbreviations: ApoE KO, ApoE Knockout; WD, western diet; BRBHT, Bok Ryung Ban Ha-Tang; T-chole, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Values are expressed as the mean ± SEM. n=8; ***P < 0.001, vs. ApoE KO mice without WD (Con; Normal diet). **P < 0.01, vs. Western diet-fed mice (Con; Normal diet).

Effect of BRBHT on biochemical levels

The serum biochemical parameters are listed in Table 4. The serum levels of total cholesterol (P < 0.001), Glucose (P < 0.01), AST (P < 0.001) and ALT (P < 0.001) were significantly elevated in mice fed WD compared with control group, while also significantly decreased (P < 0.001 and 0.05) by BRBHT or Atorvastatin (Table 4).

Effect of BRBHT on atherosclerotic lesions and hepatic steatosis

WD fed mice exhibited a uniformly pale fatty liver and hepatomegaly, and these pathological changes were reversed by BRBHT. Administration of BRBHT eliminated excess fat accumulation in hepatic intracellular vacuoles, as determined by hematoxylin and eosin (H&E) and oil red o staining (Figure 2A and 2B). Moreover, atherosclerotic lesion revealed fatty streak lesions in the aortic root of WE fed mice, indicating early development of atherosclerotic plaque. But, the elevated aortic root lesion was reduced by BRBHT (Figure 2C). These result indicated that, BRBHT effectively improves diet-induced atherosclerosis and hepatic steatosis.

Discussion

The Korean herbal medicine, BRBHT, has been shown to prevent liver steatosis responses and moderately attenuated the increased body weight of WD-fed mice. On the other hand, the liver weights are listed in Table 2 and Figure 1C. The liver weight was higher in the WD group than in the Con group, However significantly lower by BRBHT.

Effect of BRBHT on blood pressure

we confirmed that BRBHT reduced blood pressure. During the course of the experiment, blood pressure in the WD group was significantly higher than that in the control group (117.0±5.48 vs. 93.0±1.30 mmHg, P < 0.01). In contrast, the blood pressure was significantly lower in the BRBHT groups (90.0±3.20 mmHg, P < 0.01) or in the Atorvastatin group (98.0±3.25 mmHg, P < 0.05). And, diastolic and systolic blood pressure was lower in the BRBHT or Atorvastatin (Table 3).
Anti-atherosclerotic effects of Bok Ryung Ban Ha-Tang

Currently, it is used to treat blood stasis by promoting blood circulation. In this study, we evaluated the anti-atherosclerotic properties of BRBHT.

The result of the present study demonstrated that BRBHT treatment reduced body weight, body weight gain, blood pressure, liver weight, and the serum levels of T-chole, LDL, TG, and glucose, in WD-fed ApoE KO mice, also AST, ALT were significantly chaged in the BRBHT mice compared with the levels in the WD mice. Moreover, Attenuation of the development of atherosclerotic lesions and reduced liver steatosis. These data show that BRBHT has potent hypolipidemic and anti-atherosclerosis effects. In addition, the BRBHT treatment no affect liver toxicity of BRBHT in rather due to a direct pharmacological action on the liver. These data suggest that BRBHT attenuates hypercholesterolemic atherosclerosis and this effect is associated with a decrease in serum lipid and liver steatosis.

High serum lipid levels, especially the elevated level of low-density lipoprotein (LDL), have been shown to be strongly related to the development of atherosclerosis [13]. Therefore, lipid lowering therapy significantly reduces the risk of cardiovascular disease such as atherosclerosis. In part, this improved outcome has been attributed to slowed progression of atherosclerosis consequent to a decrease in hypercholesterolemia [14]. Previous studies have suggested that ameliorated the progression of atheroma formation by significantly decreased total and LDL cholesterol levels in the entire aorta in ApoE KO mice fed the High fat diet [15]. In the present study, lipid levels resulted in significantly reduced T-chole, LDL, TG, Glucose levels bt BRBHT in ApoE KO mice fed WD with BRBHT compared ApoE KO mice fed WD. Presently, the BRBHT modulated plasma cholesterol level and it is possible that these effects may have inhibited atherosclerotic lesion and liver steatosis in ApoE KO mice fed WD.

BRBHT have been three species composed of Pinellia ternata (Thunb.) Breit, Wolfiporia extensa, Zingiber officinale approved for use. The three species composed are safe and efficient in their respective therapeutic categories with some different and similar action mechanisms. Pinellia ternata (Thunb.) Breit, which is effectively inhibited TNF-a induced NF-κB activation and fatty acid as PPAR agonist being key regulators of gene involved in lipid and glucose metabolism [16]. Wolfiporia extensa, Many previous studies have indicated that its extracts and components have a variety of biological activities such as anti-hypertonic, anti-inflammatory, anti-angiogenic effects [17-19]. Moreover, Gei ji Bok ryung -Hwan (Wolfiporia extensa

Figure 2. Effect of BRBHT on liver steatosis and atherosclerotic plaque in WD-induced ApoE KO mice. BRBHT was orally administered to mice at a dose of 150 mg/kg/day for 16 weeks. Representative images of (A) hematoxylin and eosin-stained sections (B) and oil red o of liver. (C) Oil red o stained of aorta. (magnification, ×100). ApoE KO, ApoE Knockout; WD, western diet; BRBHT, Bok Ryung Ban Ha-Tang.
composition) has been shown to reduced an atherosclerotic area in the abdominal aorta and prevent of endothelial damage/cholesterol deposition in the thoracic aorta of cholesterol-fed rabbits [20]. *Zingiber officinale* preferential effects on plasma lipids, reverse cholesterol transport, cholesterol synthesis and inflammatory status in hypercholesterolaemic rabbit [21]. It is likely that three drugs that possess similar mechanisms of action may result in an effective therapy. Results from these studies support our data and suggest that BRBHT reduced atherosclerotic lesions and cholesterol deposition, providing a potential strategy for preventing atherosclerosis.

In summary, BRBHT preventes atherosclerosis through reduced of atherosclerotic lesion and liver steatosis via regulated plasma levels. This finding may provide convincing evidence to support protective effects of BRBHT therapy on atherosclerosis and lipidemia without apparent toxic side effects markes BRBHT a promising candidate in the development of anti-atherosclerosis pharmacotherapy.

Acknowledgements

This research was supported by Project (K160-90) “Research for developing of classification criteria and management technique of Mibyeong” of the Korea Institute of Oriental Medicine.

Disclosure of conflict of interest

None.

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References


Anti-atherosclerotic effects of Bok Ryung Ban Ha-Tang


