Bofutsushosan, a Traditional Chinese Formulation, Prevents Intimal Thickening and Vascular Smooth Muscle Cell Proliferation Induced by Balloon Endothelial Denudation in Rats

Kenji OHNO, a,1) Hwa-Jin CHUNG, a,2) Ikuro MARUYAMA, b and Tadato TANI a,3)

a Department of Kampo-Pharmaceutics, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University; 2630 Sugitani, Toyama 930–0194, Japan; b Department of Laboratory and Molecular Medicine, Kagoshima University, School of Medicine; 8–35–1 Sakuragaoka, Kagoshima 890–8520, Japan; and c 21st Century COE Program, Toyama Medical and Pharmaceutical University; 2630 Sugitani, Toyama 930–0194, Japan.

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Bofutsushosan (BOF), a traditional Chinese formulation (Kampo formulation in Japanese), is widely used for patients with obesity and hyperlipidemia resulting from long-term inappropriate lifestyles. Since atherosclerosis, a lifestyle-related disease, is accompanied by an abnormal accumulation of vascular smooth muscle cells (VSMCs) in the intimal area of the artery, we investigated the preventive effect of BOF on intimal thickening. Oral administration of BOF extracts 3 d before and 7 d after balloon endothelial denudation dose dependently suppressed the intimal thickening and proliferation of VSMCs in the intimal area in rat carotid arteries. This model has a similar pathologic process to atherosclerosis and is considered to be an “accelerated atherosclerosis” model. BOF extract also dose dependently inhibited the migration of cultured VSMCs. BOF extract suppressed serum lipid levels, which are a major risk factor for atherosclerosis. These findings clarified the usefulness of BOF in cardiovascular risk-reduction therapy.

Key words atherosclerosis; Bofutsushosan; vascular smooth muscle cell; balloon endothelial denudation; intimal thickening; lifestyle-related disease

Atherosclerosis is a basic pathologic lesion of ischemic heart disease and brain infarction, and its preventive therapy for lifestyle-related diseases is important. Vascular endothelial cell injury and subsequent migration and proliferation of vascular smooth muscle cells (VSMCs) are closely involved in the initiation and progression of atherosclerosis.3) This pathology can be reproduced by evaluation of intimal thickening after stripping endothelial cells with a balloon catheter in animal carotid arteries. This animal experimental system involves endothelial cell injury, and VSMC migration and proliferation induced by various growth factors, and is known as an “accelerated atherosclerosis” model4) because of its similarity to atherosclerosis in humans.

Using this experimental model, we have been investigating the efficacy of traditional Chinese drugs5) and formulations (Kampo formulations)6,7) in the prevention of atherosclerosis. In this study, we investigated the preventive effects of Bofutsushosan (BOF, Fanfengtongshengsan in Chinese) on intimal thickening and VSMC proliferation. BOF is the major Kampo formulation used for the prevention of obesity5) hypertension, and insulin resistance.9) Several pharmacologic studies of BOF have focused on antiobesity,11) and anti-hyperglycemia in diabetes mellitus mice,12) although information regarding the preventive effects of BOF on atherosclerosis is lacking.

MATERIALS AND METHODS

Samples and Reagents Freeze-dried extract of BOF (lot no. ONO31AO) was supplied by Kanebo Ltd. (Tokyo, Japan). The common human (60 kg) daily dose of the preparation is 5700 mg. The crude drug composition and HPLC profile of BOF are shown in the legend to Fig. 1. The sources of the hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitor simvastatin (SV, positive control compound), anti-proliferating cell nuclear antigen (PCNA) monoclonal antibody (PC-10), biotinylated anti-mouse second antibody, and streptavidin-conjugated peroxidase were the same as in our previous report.9) The same Dulbecco’s modified Eagle’s medium (DMEM, Nissui Pharmaceutical Co., Ltd.), fetal bovine serum (FBS, JRH Bioscience), penicillin (Gibco BRL), and streptomycin (Gibco BRL) were used as in our previous study.13)

Animal Experiments Male Wistar rats (13 weeks old, 340–360 g body weight, Sankyo Lab. Service, Tokyo, Japan) were anesthetized with pentobarbital and balloon endothelial denudation in the left carotid artery was performed according to our previously described method.7) Briefly, the rats (n=8) were fed a normal diet containing 1% cholesterol and BOF (three doses as shown in Fig. 2, n=8) for 3 d before and then for 7 d after the injury. SV (0.83 mg/kg daily, n=8) was administered orally during the same period as the BOF treatment. The doses of BOF 950 mg/kg and SV 0.83 mg/kg are 10-fold higher than the common human daily dose.

The intimal thickening and proliferation of VSMCs in the intimal area in left carotid artery sections 7 d after denudation were evaluated histologically and immunohistochemically according to our previous method.7) The stenosis ratio, which is an index of the increase in intimal area and decrease in luminal area, was assessed by the equation shown in the legend to Fig. 2.

Serum lipids were examined in blood samples collected from the abdominal aorta 7 d after denudation. The total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and lipid peroxides (LPO) were also determined using commercial assay kits according to our previous method.7)

All animal experiments and care were conducted in conformity with the Guidelines of the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University.
Intimal Formation and VSMC Proliferation in Vivo

In the cholesterol-fed control rats, the intimal area (0.046±0.004 mm²) induced by denudation was more extensive (p<0.05) than that (0.040±0.002 mm²) in the normal diet-fed control rats (denuded). The results are similar to those in our previous report.7) Figure 2 shows that the increase in the intimal area and the stenosis ratio in the cholesterol-fed rats were dose dependently reduced by oral administration of BOF extract for 10 d. Oral administration of SV, the positive control compound, also significantly reduced the increase in the intimal area and the stenosis ratios.

Seven days after denudation, the number of VSMCs immunoreactive to anti-PCNA antibody in the intimal area was reduced by oral administration of BOF extract in a dose-dependent manner. The PCNA labeling index (Fig. 2) is used as an index of VSMC proliferation.15) At 10-fold higher than the human dose, the inhibitory effects of BOF extract (950 mg/kg) on intimal thickening and VSMC proliferation were comparable with those of SV (0.83 mg/kg), for which the inhibitory effects have been clarified in a similar balloon injury model.10) These results suggest that the inhibitory effects of BOF on intimal thickening depend on its inhibition of VSMC proliferation, which is considered to be a major factor in the pathogenesis of intimal thickening after endothelial injury.17)

Serum Lipids

As shown in Table 1, the serum total cholesterol and LDL cholesterol levels in the cholesterol-fed control (denuded) group after a total of 10 d of feeding were significantly (p<0.05) increased compared with those in the normal diet-fed control group. There were no significant differences in serum LPO levels between the two control groups (data not shown).

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RESULTS AND DISCUSSION

Body Weight and Food Intake

No rats (n=8) died in any experimental group over the 10 d. No abnormality of body weight or food intake was recognized. The body weight ratio on the 10th day in the BOF group (maximum dose 1425 mg/kg; 15-fold higher than the common human daily dose) was 98.1±2.4% of that on the first day.

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Each value represents the mean±S.D. (n=8). TC, total cholesterol; LDL C, low-density lipoprotein cholesterol; HDL C, high-density lipoprotein cholesterol. BOF and SV were orally administered for 3 d before and 7 d after denudation. a) Significantly different from control normal diet-fed rats (denuded) and b) from cholesterol-fed rats (denuded) at p<0.05.

Fig. 2. Effects of BOF Extract on Intimal Area, Stenosis Ratio and PCNA Labeling Index in Rat Carotid Artery 7 d after Balloon Endothelial Denudation

Each value represents the percentage (mean±S.D., n=8) of the control group denuded normal diet-fed rats. Stenosis ratio (%)=(intimal area)×100/(intimal area+luminal area). PCNA labeling index (%)=(number of PCNA-positive VSMCs in intimal area)×100/(number of total VSMCs in intimal area). BOF extract (3 doses) was administered 3 d before and 7 d after denudation. The dose of BOF 950 mg/kg/d is 10-fold higher than the common human daily dose. SV, Simvastatin (0.83 mg/kg/d; 10-fold higher than the common human daily dose).* Significantly different from the CHO group (denuded cholesterol diet-fed rats) at p<0.05.

VSMC Migration in Vitro It has recently been considered that not only lipid deposition but also endothelial cell injury-induced VSMC migration and proliferation play important roles in the development of intimal thickening in atherosclerosis. Inhibition of VSMC migration and proliferation may lead to the development of a preventive drug for atherosclerosis. Thus we investigated the effects of BOF on the migration of cultured VSMCs, and found that direct addition of BOF extract (25—250 μg/ml) inhibited VSMC migration in a dose dependent manner (Fig. 3). The concentration was rather high in this experiment, but the viability of VSMCs treated with BOF extract (100 and 250 μg/ml) was 97.4±1.7 and 96.6±0.7%, respectively. From these results, it is suggested that the inhibitory effects of BOF extract on VSMC migration are not directly involved in cell death.

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Aspects of chronic inflammatory disease\textsuperscript{22} associated with reactive oxygen species and various cytokines.\textsuperscript{23} Thus BOF containing Rhei Rhizoma\textsuperscript{24} and Scutellariae Radix\textsuperscript{25} may have atherosclerosis-preventive effect. The inhibitory actions of a Rhei Rhizoma component combined with BOF on nitric oxide\textsuperscript{20} and superoxide anion,\textsuperscript{27,28} the free radical-scavenging action of Scutellariae Radix,\textsuperscript{29} and the scavenging actions of Angelicae and Paoniae Radix on the superoxide anion and hydroxyl radical\textsuperscript{30} are also considered to be involved in the inhibitory effects of BOF on intimal thickening.

In conclusion, the effects of BOF, which is widely used in the prevention of lifestyle-related diseases, on intimal area formation associated with VSMC migration and proliferation were investigated. Continuous administration of BOF for 10 d prevented intimal thickening after balloon endothelial denudation in rat carotid arteries. This action was associated with inhibition of VSMC migration and proliferation and improvement of serum lipid levels (total cholesterol and LDL cholesterol). The details of the mechanism of action of BOF remain to be analyzed, but the results of this study are pharmacologic evidence for the usefulness of BOF in the prevention of lifestyle-related diseases.

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textbf{REFERENCES AND NOTES}

1) Present address: Department of Frontier Japanese-Oriental (Kampo) Medicine, Graduate School of Medicine, Chiba University, 1–8–1 Inohana, Chuo-ku, Chiba 260—8670, Japan.

2) Present address: Department of Pharmacognosy, College of Pharmacy, Ewha Womans University, 11–1 Daehyun-dong, Seodaemun-gu, Seoul, 120—750, Korea.


