Plant Res. 4(3) 189~191(2001)

Development of New Bioproduct for Prevention of Vascular Disease from Plant Resources

Dong-Wook Kim
Intractable disease research center, Dongguk University, Sukjang-Dong, 707, Kyungju City, Kyungpook 780-714

Artheriosclerosis is a group of disease characterized by the thickening of the artery wall and in the narrowing of its lumen. It is most prominently found in the disease, atherosclerosis. Atherosclerosis is a type of arteriosclerosis that affects large arteries, and the underlying pathologic condition in most cases of coronary heart disease, aortic aneurysm, peripheral vascular disease and stroke. Athereosclerosis is caused by high blood plasma concentrations of low density lipoproteins, LDL. LDL contains specific functional groups which allow it to be recognized by most cells in the body and remain soluble in blood plasma. Therefore LDL readily passes through the endothelium, contributing to the development of plaques, atheromas.

Steinberg *et al.* has demonstrated that oxidized low-density lipoprotein (Ox-LDL) is specifically incorporated by macrophages, resulting in the formation of foam cells. Oxidative modification of LDL is causally involved in the initiation and promotion of atherosclerosis. Some of the naturally occurring substances, particularly those present in the diet, are known to inhibit LDL oxidation, and to prevent cardiovascular diseases. From this viewpoint, nutrition and diet seem to have an important role in preventing the development of atherosclerosis.

Apocynum *venetum* L., Luobuma in Chinese, has been used in traditional Chinese medicine for the prevention and treatment of hypertension, bronchitis and common cold. In the northern provinces of China where Luobuma grows wild, it is the custom to drink Luobuma tea for clearing heat and easing dizziness in terms of traditional Chinese medicine. We demonstrated that Luobuma extract administered orally to rats fed a high-cholesterol diet decreased the level of cholesterol in the blood and improved the atherosclerosis.

For the effects of Luobuma against oxidation of LDL in cell culture system, we have performed both endothelial cell injury and foam cell formation of macrophages. LDL oxidized by Cu ion, leading to peroxidation of endothelial cells. The peroxidation was suppressed when Lubuoma extract was present in the incubation mixture. As the concentration of Luobuma extract increased in the culture medium, the levels of thiobarbituric acid reactive substance (TBARS) decreased markedly. When endothelial cells were incubated in the presence of LDL and $CuSO_4$, the release of LDH was about 2.1 times higher than that in the absence of $CuSO_4$, providing evidence of cell injury by copper ions. However, the LDH release in Luobuma-treated cells was significantly decreased at both the 10 and 100μg/mL, the latter value being 41% lower than the control. In addition, Luobuma extract was dose dependently prevented against the cytotoxicity induced by copper ion in endothelial cells. *In vivo* system, peroxidation was found to be significantly reduced when LDL isolated from the plasma of rats treated with

Corresponding author : **Dong-Wook Kim,** Intractable disease research center, Dongguk University, Sukjang-Dong, 707, Kyungju City, Kyungpook 780-714

Luobuma orally was present in the incubation medium in comparison with plasma from untreated rats. A. *venetum* extract showed considerably strong suppression of the peroxidation induced by copper.

For the purpose of identifying anti-LDL oxidative substances in the A. *venetum* extract, we examined the effects of its constituents on the LDL oxidation in the cell-free system. After incubation of LDL with Cu^{2+}, TBARS and conjugated diene formations were appreciably increased. However, the Cu^{2+}-induced oxidation of LDL was significantly inhibited by addition of either aqueous extract or constituents from A. venetum leaves. Similarly, all the isolated compounds strongly inhibited the TBARS formation. Of these, chlorogenic acid markedly inhibited it even at a low concentration of 0.1μg/mL and the inhibition was over 90% at 2-5μg/mL. IC50 of isolated compounds, whose values were in a range 1.9-23.3μM. In addition, the aqueous extract and its constituents effectively prolonged the lag time of the conjugated diene formation in the Cu2+-induced LDL oxidation. All the isolated compounds prolonged the lag time, compared with the control. Of these, catechin appreciably prolonged the lag time (t=337 min), compared with the control (t=182 min). Other constituents formed no conjugated diene within 700 min under the experimental conditions. From these results, we suggest that the Luobuma increase LDL resistance to oxidation, decreasing the consumption of endogenous antioxidants, and administration of the Luobuma may be of benefit in the prevention of atherosclerosis and cardiovascular diseases.

Percutaneous transluminal coronary angioplasty (PTCA) is one of the therapeutic option in the treatment of patients with atherosclerotic coronary artery disease. However, this therapeutic method remained a problem occurring of restenosis within the first 6 months after the procedure in over 57% of patients. The exact mechanism of its process is not well understood, however, it is known major components are related to thrombosis and migration and proliferation of smooth muscle cells (SMCs).

Many pharmacological investigations to prevent the restenosis after PTCA have been made, however, the majority of clinical studies to reduce the incidence of restenosis using any single pharmacological agent have failed. Therefore, in order to search preventive drugs against the restenosis after PTCA from the TCFs, we have started to examine inhibitory effect of 8 TCFs on intimal thickening in rat carotid artery injured by balloon endothelial denudation. Among the 8 TCFs examined, Hwangryeunhaedok-tang (HHT), Chodung-san (CDS), Shihogayonggolmoryeu-tang (SGYMT) and Dasunggi-tang (DST) significantly inhibited intimal thickening 7 days after denudation. The 4 TCFs also inhibited vascular smooth muscle cells (SMCs) proliferation, which is considered to be a major factor in the development of restenosis after percutaneous transluminal coronary balloon angioplasty (PTCA). Present results suggest that further evaluation of the 4 TCFs as an inhibitor of SMCs proliferation to prevent arteriosclerosis is warranted. Among them, SGYMT markdly inhibited the intimal thicking and SMC proliferation in intima than the other TCF. Therinforth, we are performed to evaluate the efficacy of SGYMT on neointimal formation and intimal SMCs proliferation after balloon injury.

Eight weeks after endothelial injury, neointimal thickening was observed in the subendothelial layers the vehicle group. The SGYMT at doses of 400, 800 and 1200 mg/kg/day were orally administered 7 days before endothelial injury, and administration continued until the animal removed rat carotid artery. Intimal thickening tended to be reduced in animal receiving SGYMT at a dose of 400 mg/kg SGYMT compared with vehicle rat. However, SGYMT vessel sections administered 800 and 1200 mg/kg were distinctly decreased compared with the vehicle vessel section.

The rations of the intimal area to medial area (I/M ratio) in the SGYMT-treated groups(400, 800, 1200 mg/ml) were 1.14± 0.08, 1.01± 0.05 and 0.85± 0.05, respectively. The values were significantly decreased ($p<0.01$, 400 mg/kg: $p<0.001$, 800 and 1200 mg/kg) compared with vehicle group (1.44±0.09). The inhibition of cell proliferation in the rat carotid artery administered SGYMT-treated groups was studied by immunohistochemical method, using PCNA monoclonal antibody. The proliferative action of SGYMT was confirmed by determining the total number of cells, PCNA positive number of cells and the PCNA labeling index. The total number of cells (PCNA positive plus negative cells) within intimal areas in SGYMT-treated groups did not changed statistical significance compared with vehicle group. In PCNA positive number of cells in SGYMT at doses of 400, 800 and 1200 mg/kg/day were 43.7±5.7, 58.8±12.4 and 40.3±7.3, respectively (control, 104.0±13.0). Whereas, PCNA labeling indics were significantly lower than vehicle group. These finding indicate that SGYMT shows a higher inhibitory potency on intimal formation, due to inhibition of intimal smooth muscle cells.

REFERENCES

Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., and Witztum, J. L. 1989. Beyond cholesterol. Modification of low-density lipoprotein that increases its atherogencity. *N. Eng. J. Med.* **320**, 915-924.

Kim, D. W., Yokozawa, T., Hattori, M., Kadota, S., and Namba, T. 1998. Luobuma leaf inhibits oxidation of low-density lipoprotein in cholesterol-fed rats. *J. Trad. Med.* **15**, 40-44.

Yokozawa T., Kim, D. W., Hattori, M., Kadota, S., and Namba, T. 1999. Effect of Luobuma leaves against oxidation of low-density lipoprotein: A cell culture assay. *J. Trad. Med.* **16**, 141-147.

Kim, D. W., Yokozawa, T., Hattori, M., Kadota, S., and Namba, T. 2000. Inhibitory effects of an aqueous extract of Apocynum venetum leaves and its constituents on Cu^{2+}-induced oxidative modification of low density lipoprotein. *Phytother.* Res. 14, 501-504.

Topel, E. J., Leby, Fo, Pinkerton, C. A. et al. 1993. A comparison of directional atherectomy with coronary angioplasty in patients with coronary artery disease. *N. Engl. J. Med.* **329**, 221-227.

Wei, M. J., Shintani, F., Yagi, G. and et al. 1997. Endothelium-dependent and independent vasoactive actions of a Japanese kampo medicine, Saiko-ka-ryukotsu-borei-to. *Biomed & Pharmacother*, **51**, 38-43.

Kim, D. W., Chung, H. J., Tani, T. et al. 2000. Effect of Saiko-ka-ryukotsu-borei-to on common carotid artery after endothelial denudation by balloon catheter, The 17th Society of Japan Traditional Medicine. 75 P