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 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 1.9-23.3 μM. 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.