Abstract

Cardiovascular diseases, developing as a result of atherosclerosis progression, are the leading cause of death worldwide and generate annually billions of costs in health and social care systems. The Rotterdam studies conducted in 1990s, showed a negative correlation between vitamin K2 (VK2) intake and coronary heart disease mortality. VK affects vascular system not only through modulation of blood clotting but also inhibiting vascular calcification which concomitants atherosclerosis.

The main objective of this studies was to determine the effect of VK in development and progression of atherosclerosis, with particular regard to the long-chain menaquinon-7 (MK-7) administrated in synthetic form as well as food product (natto). Hypothesis about antiatherosclerotic action of VK2 was verified through three nutritional experiments on animal model of atherosclerosis – ApoE/LDLR-/- double knockout mice. In the experiment I eightweek supplementation of selected forms of VK (VK1, MK-4 i MK-7) in 10 mg/kg b.w. was applied to mature (24-week old) ApoE/LDLR-/- mice. Next experiment was conducted on growing (16-week old) animals supplemented with in 0,05 mg and 1 mg/kg b.w. doses. In the experiment III soybeans seeds were fermented by Bacillus subtilis natto bacteria to obtain product with high VK2 content which was then administrated to growing ApoE/LDLR-/- mice during eight-week period. Amount of fermented soy addition was adjusted to the doses of MK-7 in the experiment II.

Analysis performed in the experiment I demonstrated significant effect of VK2 on body weight reduction. Additionally, beneficial effect of VK was shown in regard to lipid metabolism manifested by lower values of TC/HDL-C ratio. On the basis of histological analysis diverse impact of MK-4 and MK-7 on atherosclerosis progression in aortic roots was found. Based on results from the experiment I MK-7 was chosen for further studies. In the experiment II significant effects of MK-7 administrated in dose 1 mg/kg b.w. on lipid metabolism were observed. This supplementation had hypocholesterolemic effect significantly reducing total and LDL plasma cholesterol concentration. In adipose tissue from mice receiving high dose of MK-7 content of oleic acid was significantly lower and content of saturated fatty acids higher compared to animals receiving 0,05 mg/kg b.w. dose. Moreover, increase in odd fatty acids was found. Similar effect was found in liver fat (for C17:0 acid) where additionally occurred reduction in the values of de novo lipogenesis index. Histological analysis revealed only slight reduction in atherosclerotic plaque area in aortic roots whilst an ambiguous effect of MK-7 on area and plaque structure in whole aorta and brachiocephalic artery (BCA).

In the experiment III significant effect of soybean fermentation on chemical composition of seeds manifested by increase in moisture, crude protein and fat was confirmed. Fermentation increased also total polyphenols content however with no effect on antioxidant capacity measured by TEAC method. In in vivo study feeding mice with fermented soybean natto had no effect on body weight, lipid metabolism and antioxidant capacity of plasma and liver tissue. Nonetheless significant inhibition of atherogenesis was found in BCA proximal section as a result of administration diet containing 19% w/w natto. At the same time no effect of natto feeding on inflammation severity (assessed basing on MOMA-2 positive area) was found in distal section of examined artery.

In conclusion, selected forms of VK have diverse biological effects, dependent on length and unsaturation degree of naphthoquinone side chain. Results from performed studies do not confirm hypothesis about antiatheroscerotic effect of synthetic VK2 supplementation. However, a significant effect of VK2 on body weight and lipid metabolism in ApoE/LDLR-/- mice was demonstrated. These effects did not occur after feeding with food-derived VK2 in form of natto. Despite this increased natto consumption had antiatherosclerotic effect in BCA proximal section of growing ApoE/ LDLR-/- mice