Atherosclerosis is a multietiological inflammatory and degenerative vascular disease with growing incidence in westernized populations. Pathomechanism and treatment of this disease have been extensively studied on animal models for last decades. Recently, gene-targeted apolipoprotein E and LDL receptor-double knockout (apoE/LDLR-DKO) mice have been engineered, representing a new murine model that displays severe hyperlipidemia and atherosclerosis. We have successively used apoE/LDLR-DKO mice to study biological effects of new antiatherosclerotic drugs and diets [1,2]. Furthermore, we applied synchrotron radiation microprobes to characterize elemental composition of atheromas in this animal model [3]. The aim of the present study was to show changes in the distribution of selected elements in atherosclerotic plaques of apoE/LDLR-DKO mice fed egg-rich proatherosclerotic diet supplemented or not with antiatherosclerotic drug perindopril. We have combined synchrotron radiation micro-XRF with histological stainings to determine distribution and concentration of the elements in histologically defined areas of atherosclerotic lesions.

Fifteen female apoE/LDLR-DKO mice were used for the study. Up to the age of 2 months the mice were fed a commercial, cholesterol-free pelleted diet and then they were randomly assigned to one of three experimental groups fed for the following 4 months: i. AIN-93G diet (n=5; CHOW), ii. AIN-93 diet supplemented with egg-yolk lyophilisate (for details see [2], n=5; LIOPH), iii. AIN-93G diet supplemented with egg-yolk lyophilisate and perindopril (2 mg/kg b.w., n=5; LIOPH/PERIND). Six-month-old animals were sacrificed; hearts with ascending aorta were dissected out and snap-frozen. Serial 10 µm-thick crossections of the aortic root were cut on cryostat and mounted either on poly-L-lysine coated slides (histology) or on 3 µm-thick Mylar foil (microprobe). Consecutive slides were stained with oil red O (ORO; red) for the demonstration of lipids and double immunostained: CD68 for macrophages (green) and smooth muscle actin (SMA) for smooth muscle cells (red).

All micro-XRF measurements were carried out at beamline L of the storage ring DORIS III. The primary photon energy was set to 17.5 keV by a multilayer double monochromator. A polycapillary half-lens was used for beam focusing, hence the final beam size on the sample was approximately 15 µm in diameter. Emitted elemental spectra were recorded with Vortex SDD detector. Two-dimensional maps were acquired from lesional areas of the aortic root with surrounding cardiac muscle (resolution 15 µm, time of acquisition 5 s from each point). From morphologically defined areas, precise point spectra were recorded (resolution 15 µm, time of acquisition 300 s). The results were normalized to beam current, thickness of sample and time, and expressed in arbitrary units (mean ± SD).

Based on histological stainings, more advanced atherosclerosis expressed by total area occupied by lipids, number of macrophages and smooth muscle cells was observed in animals fed egg-rich diet. The perindopril treatment slightly attenuated these effects (see figures below). In animals fed egg-rich diet, higher concentrations of Ca, P, K and lower concentrations of Cl, Cu, Fe, Se, Zn in atheromas were seen in comparison to chow diet-fed animals. After perindopril treatment, concentrations of Ca, Cl, Cu, K, Se and Zn showed the tendency to achieve levels like in chow diet-fed animals.
Figure 1: Relative concentrations of selected elements in atheromas of control group (CHOW), animals fed egg-rich diet (LIOPH) and mice fed egg-rich diet including perindoprilat (LIOPH/PERIND).

Figure 2: Histological stainings (ORO; CD68/SMA) of aortic roots of mice from groups LIOPH and LIOPH/PERIND. Distributions of Ca and Zn in corresponding areas (marked yellow on ORO pictures).

References

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