DISTRIBUTION OF SELECTED ELEMENTS IN ATHEROSCLEROTIC PLAQUES OF APOE/LDLR-DOUBLE KNOCKOUT MICE SUBJECTED TO DIETARY AND PHARMACOLOGICAL TREATMENTS

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Atherosclerosis is a multietiological inflammatory and degenerative vascular disease with growing incidence in westernized populations. Gene-targeted, apoliporotein E and LDL receptor-double knockout (apoE/LDLR-DKO) mice represent a new animal 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 perindoprilat (inhibitor of angiotensin converting enzyme). We have combined synchrotron radiation micro-XRF spectrometry with histological stainings to determine distribution and concentration of trace and essential elements in histologically defined areas of atherosclerotic lesions.

Fifteen female apoE/LDLR-DKO mice were used for the study. Up to the age of 4 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 2 months: *i.* AIN-93G diet (n=5; CHOW), *ii*. AIN-93 diet supplemented with 5% egg-yolk lyophilisate (*n* = 5; LIOPH), *iii*. AIN-93G diet supplemented with egg-yolk lyophilisate and perindoprilat (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 cryosections of the aortic root were cut and mounted either on routine slides (histology) or on 3 μm-thick Mylar foil (microprobes). Consecutive slides were stained with oil red O (ORO) for the demonstration of lipids, picrosirius red for collagen fibers and double immunostained: CD68 for macrophages and smooth muscle actin (SMA) for smooth muscle cells.

All micro-XRF measurements were carried out at beamline L of the storage ring DORIS III (Hasylab, Hamburg). 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).

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 perindoprilat treatment slightly attenuated these effects. 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 perindoprilat treatment, concentrations of Ca, Cl, Cu, K, Se and Zn showed the tendency to achieve levels like in chow diet-fed animals.

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