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

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Introduction

Atherosclerosis is a multietiological inflammatory and degenerative vascular disease with growing incidence in westernized populations. Pathomechanism and treatment of atherosclerosis have been extensively studied on animal models for last decades. Gene-targeted, apolipoprotein 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].

Goal of the study

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.

Materials and methods

Fifteen female apoE/LDLR-DKO mice were used. 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:
a) AIN-93G diet (n=5; **CHOW**),
b) AIN-93 diet supplemented with 5% egg-yolk lyophilisate (n=5; **LIOPH**),
c) 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 cross-sections of the aortic root were cut frozen 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 and double immunostained: **CD68** for macrophages and smooth muscle actin (**SMA**) for smooth muscle cells.

XRF examination

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 types of recordings were performed:
a) two-dimensional maps were acquired from areas of the aortic root with surrounding cardiac muscle (resolution 15 μm, time of acquisition 5 s from each point),
b) precise point spectra were recorded from morphologically defined areas, (resolution 15 μm, time of acquisition 300 s).
The results were normalized to beam current, thickness of the sample and time, and expressed in arbitrary units (mean ± SD).

References

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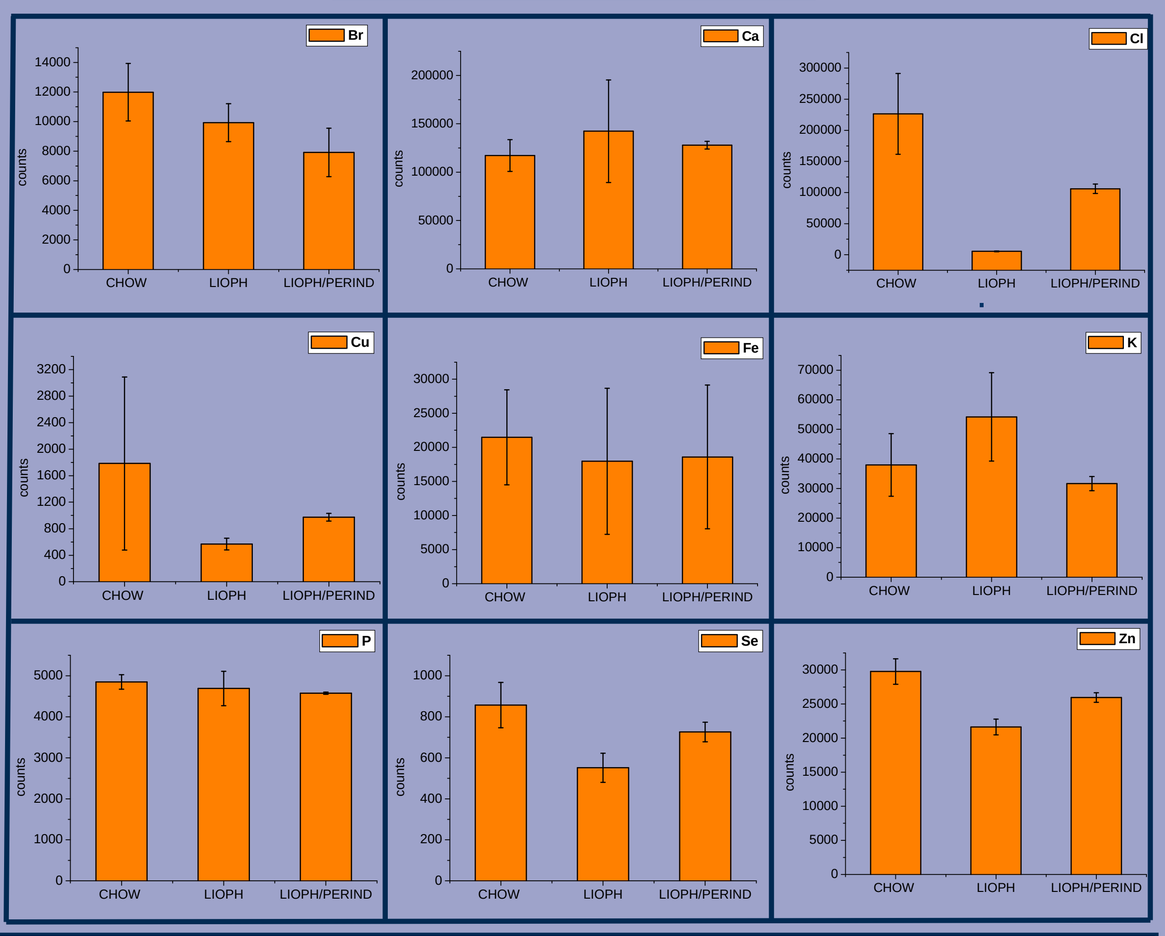
Acknowledgements

The study was supported by contract RII3-CT-2004-506008 (IA-SFS) of the EU Commission. The data were obtained during realization of DESY-D-I-20090125 and D-II-20100089EC projects.

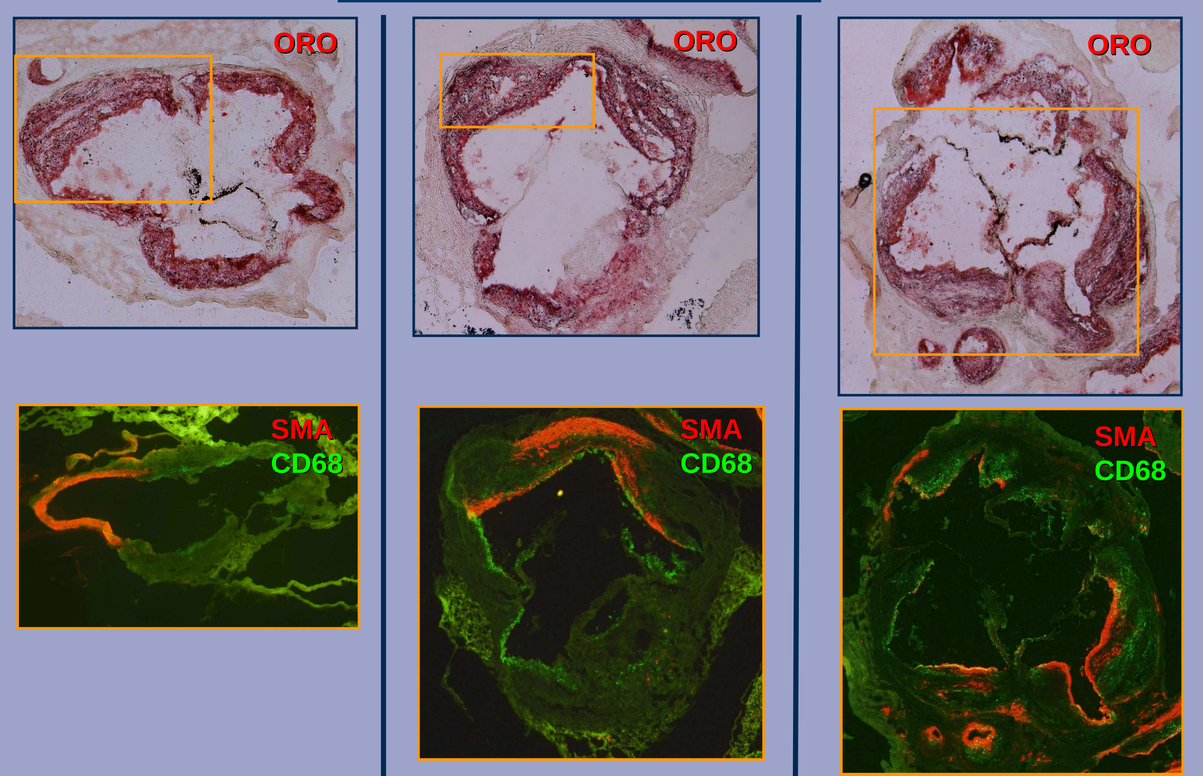
Results

Point analysis



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.

2D maps



CHOW 1005 x 615 μm^2 | **LIOPH** 1005 x 510 μm^2 | **LIOPH/PERIND** 1095 x 1515 μm^2