EFFECT OF AVE 0991 – ANGIOTENSIN-(1-7) RECEPTOR AGONIST TREATMENT ON ELEMENTAL AND BIOMOLECULES DISTRIBUTION IN ATHEROSCLEROTIC PLAQUES OF APOE-KNOCKOUT MICE

J. Kowalska[1*], **M. Gajda**[2], **W.M. Kwiatek**[1], **K. Appel**[3], **and P. Dumas**[4]

[1] *Institute of Nuclear Physics, Polish Academy of Sciences, Radzikowskiego 152, 31–342 Kraków, Poland*
[2] *Department of Histology, Jagiellonian University Medical College, Kopernika 7, 31–034 Kraków, Poland*
[3] *Hasylab, Deutsches Elektronen Synchrotron, Notkestraβe 85, D–22607 Hamburg, Switzerland*
[4] *SOLEIL, L'Orme des Merisiers, Saint Aubin – BP 4, 91–192 Gif-sur-Yvette CEDEX, France*

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[*] *e-mail*: joanna.kowalska@ifj.edu.pl

Atherosclerosis is a multietiological inflammatory disease involving large and medium-sized arteries with growing incidence in westernized populations. Despite of many intense studies the patomechanism of this disease is still not fully understood. Gene-targeted, apoliporotein E – knockout (apoE-KO) mice display early and advanced vascular lesions (including lipid deposits and inflammatory cell infiltratrion as well as valvular calcifications), therefore became a reliable model to investigate the patomechanism and treatment of this disease.

The aim of the present study was to investigate the changes in the distribution of selected pro- and anti- inflammatory trace elements as well as biomolecules in atherosclerotic plaques of apoE-KO mice fed chow diet supplemented or not with AVE 0991 — angiotensin-(1-7) receptor agonist (a potential candidate for atherosclerosis treatment). In this study both synchrotron radiation based micro XRF (X-ray Fluorescence) and micro FTIR (Fourier Transform InfraRed) spectroscopy were applied and combined with histological stainings.

Based on histological staining more advanced atherosclerosis expressed by total area occupied by lipids was observed (oil-red-O staining). Moreover higher enzymatic activity and larger area by metalloproteinases was observed in the control group of animals as compared to the AVE 0991 treated ones (zymographically with fluorescein-gelatin) [1].

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 and the beam was focused to the final size of 15 μm in diameter using a polycapillary half-lens. All emitted spectra were recorded with a Vortex SDD detector. Two types of measurements were performed:

a) 2D maps acquired from lesional areas of aortic root with surrounding cardiac muscle (15 μm step, 5 s/point),

b) precise point spectra from morphologically defined areas (15 μm resolution, 300 s/point) [2].

The distribution of P, Cl, Ca, Fe, Cu and Zn corresponded well with histological structures of the atherosclerotic lesions. Iron as a proatherogenic element appeared mostly in the region of atheroma as compared to other tissue structures and there was no significant difference in the concentration of this element in both studied groups. On the other hand Zn a hypothetically antiatherogenic element occurred mainly in peripheral regions of the aortic cross section and its concentration was higher in the control group as compared to the AVE 0991 treated one. Similar observation were seen in case of Cu distribution and concentration. Some spots of high Ca and P concentration (both considered as a relatively late event in the progression of atherosclerosis) were visible in the area of high lipids deposition.

Micro-FTIR measurements were performed at SMIS beamline at synchrotron SOLEIL facility equipped with a Nicolet Nexus 5700 spectrometer coupled with a Continuum XL IR microscope and a 50 μm MCT/A detector. All spectra were taken in transmission mode using a 12 μm FWHM beam size, with a 4 cm^{-1} spectral resolution and repetition of 128 scans in order to get a good statistics. Two types of measurements were performed like in case of XRF analysis.

The biomolecular analysis was mostly focused on the phospholipids saturation level (2930 cm^{-1} / 2960 cm^{-1}) and lipid to protein ratio (1730 cm^{-1} / 1660 cm^{-1}). Both of this parameters were lower in case of AVE 0991 group as compared to the control one, which may imply the influence of the applied pharmaceutical on the lipid oxidation process in atherosclerosis. Moreover, the protein secondary structure was studied based on the composition on the amide *I* band (1660 cm^{-1}) demonstrating the higher percentage content of β-sheet structure as compared to the α-helix one in both groups and also some switches in the bands positions, which may indicate the changes in protein structure.

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