Scientific Report (December 2019 - October 2020)

Project PN-III-P1-1.1-PD-2016-1942

"Evaluation of the therapeutic potential of non-viral apolipoprotein E gene transfer to limit progression of atherosclerosis"

In the period December 2019 - October 2020, in the project PN-III-P1-1.1-PD-2016-1942 entitled: „Evaluation of the therapeutic potential of non-viral apolipoprotein E gene transfer to limit progression of atherosclerosis", the experiments planned in the activity A3.1: „Evaluation of atherosclerotic plaques after systemic administration of apoE polyplexes in apoE-deficient mice" were performed.

Apolipoprotein E (apoE) has anti-atherosclerotic properties (facilitates the cellular cholesterol efflux) as well as anti-inflammatory and antioxidant properties. The majority of plasma apoE comes from the liver, but apoE is also synthesized by macrophages, spleen, lungs, kidneys, muscles and brain. The biological activity of apoE may be influenced by changes in its structure or quantity. A structural alteration is generated by the polymorphism of the APOE gene located on the long arm of chromosome 19, being described three alleles (ε2, ε3 şi ε4) encoding three isoforms: E2, E3, and E4, which differ in the amino acids present at positions 112 and 158. The frequency of the three alleles in the Caucasian population varies as follows: ε2 = 8-12%, ε3 = 74-78%, ε4 = 14-15%. E3 isoform is associated with normal lipid metabolism. ApoE2 has a low affinity for the LDL receptor, which leads to higher plasma levels of apoE, and in response, the liver increases the expression of LDL receptors resulting in lower cholesterol levels. Unlike apoE2, apoE4 has a more effective "clearance", which leads to lower plasma levels of apoE and higher cholesterol levels. In addition, apoE4 has been associated with Alzheimer's disease. ApoE deficiency is associated with atherosclerosis in both humans and mice. Systemic overexpression of apoE in mice induces hypertriglyceridemia, indicating that an optimal level of apoE protein in plasma is required for proper cholesterol removal.

In this project, we administered C60PEI-apoE3 polyplexes to atherosclerotic mice as a therapeutic agent to reduce the atherosclerotic process. In the first stage of the project, we obtained transfection agents based on C60-PEI polyplexes with apoE3 encoding DNA. Using molecular cloning methodology, we obtained five constructs containing apoE3 encoding DNA (the total sequence or several apoE fragments cloned into the pcDNA3.1-DYK vector) required for *in vivo* transfection. In the second stage of the project, the functionality of C60-PEI-apoE3 nanoparticles as *in vivo* transfection agents was tested after injections of mice with C60-PEI polyplexes and apoE3 encoding DNA. In the third stage of the project, atherosclerotic plaques were evaluated after systemic administration of C60-PEI-apoE polyplexes to apoE-deficient mice. ApoE-deficient mice were treated with a hypercholesterolemic diet throughout the period of administration of C60-PEI-apoE polyplexes. The amount of apoE protein in the serum of apoE-deficient mice treated with polyplexes was determined using the "Human ApoE ELISA development kit". For the "en face" evaluation of the atherosclerotic plaques, the mice's aorta were fixed with 4% PFA and stained with Oil Red O. To avoid using an excess of C60-PEI *in vivo*, the formation of polyplexes was optimized using DNA sequences encoding three fluorescent proteins (A7TagBFP, A8Cerulean or B7PA-GFP cloned in SalI and Eco47III sites of pEYFPC1 vector).

Publications:

- *The Opposite Effect of c-Jun Transcription Factor on Apolipoprotein E Gene Regulation in Hepatocytes and Macrophages,* Violeta G. Trusca, Elena V. Fuior, Dimitris Kardassis, Maya Simionescu, Anca V. Gafencu*, Int J Mol Sci. 2019 Mar 23;20(6):1471, PMID: 30909560*
- *The Mechanism of Bisphenol A Atherogenicity Involves Apolipoprotein A-I Downregulation through NF-κB Activation,* Violeta G. Trusca, Madalina Dumitrescu, Ioana M. Fenyo, Irina F. Tudorache, Maya Simionescu, Anca V. Gafencu*, Int J Mol Sci. 2019 Dec 12;20(24):6281, PMID: 31842455*
- *Manuscript in preparation: Fullerene-based nanoparticles conjugated with apolipoprotein E encoding DNA used in the anti-atherosclerosis therapy,* Violeta G. Trusca, Ioana M. Fenyo, Mădălina Dumitrescu, Cristina Uritu, Mariana Pinteala, Anca V. Gafencu.