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Background and Aims: ROR-alpha is a member of ligand-activated nuclear receptor superfamily of transcription factors. Melatonin and cholesterol are two known ligands of ROR-alpha, both of which were shown to be important for atherosclerosis. The aim of this study was to determine differentially expressed miRNAs in coronary artery disease (CAD) patients, and to test the dependence of their expression on ROR-alpha activity in THP-1 macrophage cells.
Methods: The Agilent's microarray analyses were performed to compare plasma miRNA profiles of selected individuals with CAD (n=4) and non-CAD (n=3). Promo transcription factor binding site prediction tool was used to identify ROR-alpha response elements (ROREs) in promoter of selected miRNAs genes. Furthermore, THP-1 macrophage cells were treated with simvastatin and ROR-alpha specific ligands (CPG52608 and SR1001) and the expressions of differentially expressed miRNAs were analyzed.
Results: Microarray results indicate that miR-19a and miR-26a were significantly up-regulated in CAD patients compared to non-CAD group (fold change>1.5, p<0.05). We identified ROREs within the promoter regions of miR-19a host gene *MIR17HG* and miR-26a host gene *CTDSP2/CTDSPL*. In addition, we observed that simvastatin repressed the expression of miR-19a and miR-26a, and this repression was partially increased by SR1001 for miR-26a in macrophage cells. While repressor effect of simvastatin was more clear in miR-26a, its prevention by CPG52608 was observed to be more effective in miR-19a and miR-26a.
Conclusions: Our results suggest that miR-19a host gene *MIR17HG* and miR-26a host gene *CTDSP2/CTDSPL* are potential target genes of ROR-alpha. It seems also that ROR-alpha may control various genes indirectly through miRNA clusters.

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02. Inflammation, immunity and macrophages - 02.02 Macrophages in lipid metabolism and atherosclerosis

EAS19-0693.
EZETIMIBE PROTECTS THP-1 CELLS FROM ISCHEMIA-REPERFUSION INJURY REDUCING OXIDATIVE STRESS AND UP-REGULATING NRF2/ARE GENE EXPRESSION

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Background and Aims: We demonstrated that physical training, characterized by repeated ischemia-reperfusion (I-R) episodes (ischemic conditioning, IC), protects circulating cells from peripheral artery disease (PAD) patients against ischemic harms by reducing oxidative stress (OS) and by up-regulating nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway expression. Ezetimibe (Eze) has been shown to alleviate OS enhancing Nrf2 nuclear translocation in an AMPK/p62-dependent manner. In a cellular I-R and IC model, we aimed to investigate: 1) the effect of Eze on OS and Nrf2/ARE gene expression 2) whether Eze could have a synergistic effect on IC.
Methods: THP-1 cells were treated with or without Eze (50μM) overnight, then subjected to 1 or 6 repetitive I-R cycles using EVOS FL Auto Imaging System. Reactive oxygen species (ROS) formation was evaluated with DCF in cytofluorimetry. Nrf2/ARE and p62 gene expression were evaluated by RT-PCR and western blotting.
Results: When THP-1 cells were exposed to 1 I-R cycle, the preincubation with Eze significantly reduced ROS formation (p<0.01) and up-regulated Nrf2/ARE pathway expression and p62 phosphorylation (p<0.001). Multiple I-R cycles, acting as IC, significantly reduced ROS formation and up-regulated Nrf2/ARE gene expression (p<0.001); in these conditions, Eze preincubation was able not only to almost abolish ROS formation (p<0.01) but also further up-regulate Nrf2/ARE expression.
Conclusions: In our I-R model, Eze not only restores I-R-induced oxidative damages through Nrf2/ARE signaling up-regulation but also has a synergistic effect on IC. This new "pleiotropic" effect, if confirmed in vivo, may strengthen the use of Eze in PAD patients.

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EAS19-0741.
DEVELOPMENT OF TRANSGENIC MOUSE MODEL SUPERPRODUCER SEALIDASE WITH SECRETION INTO THE BLOOD

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Background and Aims: Create a mouse model with inducible overexpression of neuraminidase (sialidase) in the blood. Neuraminidase (sialidase) is an enzyme that performs the desialylation of low density lipoproteins. Increased sialidase activity is observed in the blood of patients with atherosclerosis.
Methods: To obtain the superproducer of neuraminidase, we plan to use the *Mus musculus Neu3* gene cDNA with a secretion signal from the cell, which will be inserted into the vector with a stop tape surrounded by *loxP* sites under the b-actin promoter. Genetic construction involves the random insertion of the transgene. The export sequence of the albumin export site, 1 to 18 amino acids of the N domain of albumin, will be used as a secretion signal to the blood. Tissue-specific *lysozyme M* promoter will provide *Neu3* expression in macrophages / neutrophils after crossing recombinase-producing *Cre* mice with *Neu3* mice with a stop tape surrounded by *loxP* sites. Primary transgenes will be obtained by microinjection of the genetic construct into the pronucleus of the ovum.
Results: After crossing the obtained line of transgenic animals with a transgenic line of mice expressing the *Cre* recombinase under the *lysozyme M* promoter, we obtain the line mouse superproducers of neuraminidase expressed in the blood.
Conclusions: Our approach will allow investigating the role of free sialidase in the blood for the development of atherogenesis, including against the background of the *Apoe* mutation - -. This work was sapportid by grand RSF 17-75-20249

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02. Inflammation, immunity and macrophages - 02.02 Macrophages in lipid metabolism and atherosclerosis

EAS19-0800.
A NOVEL HYPOTHESIS: ERYTHROCYTE SENESCENCE PLAYS A KEY ROLE IN THE ATHEROSCLEROSIS DEVELOPMENT

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Background and Aims: Atherosclerosis is a chronic inflammatory disease of the arterial wall characterized by chronic inflammation, high blood pressure, oxidative stress, and progressive loss of cell and organ function with aging. We propose a hypothesis that the development of atherosclerosis is based on oxidative stress of erythrocytes and their senescence.
Methods: Our hypothesis is based on several facts. At the first, it is known that cholesterol content is increased in membrane of senescent erythrocytes. The second, senescent erythrocytes lose their plasticity and ability of deformation, which affects the rheological blood properties, that can injure the vessel wall. The third, macrophages are involved in all stages of atherogenesis. They can undergo polarization by shifting between M1 and M2 functional phenotypes.
Results: It is known that *efferocytosis, or ingestion of* apoptotic cells, is stimulated by *M2 macrophage* polarization and macrophage polarization toward the pro-inflammatory M1 macrophage is a major promoter to atheroma formation. It is known that *efferocytosis, or ingestion of* apoptotic cells, is stimulated by *M2 macrophage* polarization. A failure of *efferocytosis* leads to a prolongation of chronic pathology in tissue. In addition, fat-laden *macrophages* contribute to plague progression *by transforming into foam cells* in response to excess lipid deposition in arteries. We postulate that