Session 10: Changes in Ischemic Heart Metabolism

Lectures

L10.1

Energetics of cardiac myocyte in atherosclerosis

Ryszard T. Smoleński, Marta Toczek, Alicja Bulińska, Mariola Olkowicz, Magdalena Zabielska, Iwona Pelikant-Malecka, Barbara Kutryb-Zajac, Ewa M. Slomińska

Department of Biochemistry, Faculty of Medicine, Medical University of Gdansk, Debinki 1 80-211 Gdańsk, Poland
Ryszard T Smolenski <rt.smolenski@gumed.edu.pl>

Blood lipid pattern abnormalities are known to result in atherosclerosis, while less is known about changes in myocyte energetics. We used liquid chromatography/mass spectrometry (LC/MS) based approach for analysis of changes in cardiac energy metabolism in dyslipidemic, atherosclerosis prone ApoE-/-/LDLR-/- mice and wild-type controls (WT) that include proteomics, *in vivo* flux analysis of substrates stable isotopes, assessment of *ex vivo* myocardial substrate utilization and analysis of high-energy phosphates concentrations. Cardiac sensitivity to hypoxia was evaluated by exposure to reduced oxygen in breathing air. Differential analysis of cardiac proteome revealed co-ordinated increased expression of mitochondrial proteins, in particular enzymes responsible for fatty acid (FA) and branched-chain amino acid (BCAA) oxidation in ApoE-/-/LDLR-/- vs. WT. This correlated with decreased entry of glucose estimated both *in vivo* and *ex vivo* as well as increased entry of leucine into cardiac Krebs cycle. Cardiac high-energy phosphates were elevated while plasma free FA concentration was doubled in ApoE-/-/LDLR-/- mice vs. WT. Hypoxia induced profound changes of ECG ST segment and troponin T leakage in ApoE-/-/LDLR-/- but not in WT. We demonstrated profound metabolic alterations in the cardiac cells of dyslipidemic mice that paradoxically increased cardiac reliance on mitochondrial oxidation resulting in increased susceptibility to hypoxic damage.

L10.2

Endothelial profiling *in vivo*: novel approach to experimental pharmacology of endothelium

Stefan Chlopicki

Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, Kraków, Poland
Stefan Chłopicki <stefan.chlopicki@jcet.eu>

Vascular endothelium, is presently looked upon as an important autocrine/paracrine/endocrine organ that regulates number of cardiovascular functions and endothelium-targeted therapy mimicking, stimulating or reinforcing vasoprotective mechanisms of endothelium may prove useful in treatment of various diseases. There are number of pharmacotherapeutic mechanisms of endothelium and vascular wall that could be exploited therapeutically. For example, NO-based therapy targeted to the liver by V-PYRRO/NO may be an interesting approach to treat liver steatosis [1-4]. In turn, pharmacology of COX-2/PGI_2 pathway stimulated by 1-methylnicotinamide (MNA), affords anti-thrombotic, anti-inflammatory and vasoprotective activity [5-6] that may be therapeutically useful in atherosclerosis. To translate these endeavours of stimulating vasoprotective activity of endothelial mediators with pharmacological tools into therapeutics the better methods to detect endothelial dysfunction that could be successfully applied in humans are needed. We have developed MRI-based approach to study endothelial function *in vivo* [7] and successfully applied it in conjunction with biochemical endothelial profiling to characterize endothelial action of MNA *in vivo* [8] or to show that ENaC *in vivo* contributes to endothelial-dependent vasodilation in the physiological conditions and the preservation of endothelial barrier integrity in endotoxemia [9]. We hope that functional and biochemical endothelial profiling *in vivo* will provide an excellent tool to foster experimental pharmacology of endothelium in experimental animals and has a potential to be translated to patients.

References:

1. Maslak E *et al.* (2015) *Biochem Pharmacol* **93**: 389-400.
2. Kus K *et al.* (2015) *Drug Metab Dispos* **43**: 1028-1036.
3. Kus K *et al.* (2017) *Pharmacol Rep* **69**: 560-565.
4. Kus E *et al.* (2018) *Pharmacol Rep* **70**: 463-469.
5. Chlopicki S *et al.* (2007) *Br J Pharmacol* **152**: 230-239.
6. Mateuszuk L *et al.* (2016) *J Pharmacol Exp Ther* **356**: 514-524.
7. Bar A *et al.* (2016) *NMR Biomed* 1088-1097.
8. (2017) *Front Pharmacol* **8**: 183.
9. Šternak M *et al.* (2018) *Front Pharmacol* 9: 178.

Oral presentations

O10.1

Nucleotide and adenosine converting ecto-enzyme pattern in endothelial activation and vascular inflammation

Barbara Kutryb-Zajac[1], Paulina Mierzejewska[1], Alicja Bulinska[1], Agnieszka Jasztal[2], Patrycja Jablonska[1], Marcin Serocki[3], Rafa Bartoszewski[3], Ewa M. Slominska[1], Stefan Chlopicki[2], Ryszard T. Smolenski[1]

[1]Department of Biochemistry, Medical University of Gdansk, Poland; [2]Jagiellonian Centre for Experimental Therapeutics, Kraków, Poland; [3]Department of Biology and Pharmaceutical Botany, Medical University of Gdansk, Gdańsk, Poland
Barbara Kutryb-Zajac <b.kutryb-zajac@gumed.edu.pl>

Membrane ecto-enzymes control local concentrations of extracellular nucleotides and adenosine that affect vascular pathologies by mediating inflammation and lipid accumulation. We reported recently that changes in ecto-enzyme activities are related with valve dysfunction and atherosclerosis. This work aimed to investigate sources of ecto-enzymes in the vasculature during initial and later phases of atherosclerosis and to correlate their activities with markers of endothelial activation.
Histological analysis of human arteries revealed that at an early stage of pathological process ecto-nucleoside triphosphate diphosphohydrolase 1 (CD39) originated from endothelial and vascular smooth muscle cells, while ecto-5'nucleotidase (CD73) and ecto-adenosine deaminase (eADA) derived mainly from endothelium. In later stages, CD39 and particularly eADA, while not CD73 originated from immune infiltrate. The functional activities of ecto-enzymes were estimated by HPLC, while vascular expression of adhesion molecules was analyzed by PCR. eADA activity positively correlated with ICAM-1 ($r=0.69$, $p<0.05$) and VCAM-1 ($r=0.65$, $p<0.05$) expression, while CD39 and CD73 activities tended to the negative correlation with these parameters.
The activities of nucleotide converting ecto-enzymes are adversely modified during vascular inflammation and endothelial activation. They may be indicated as markers of pathological process within the vessel wall and target for the therapy in vascular diseases.

O10.2

Antioxidant activity of alcohol extracts from some plants of Armenian flora: the highest level and its possible nature

Naira Sahakyan, Margarit Petrosyan, Armen Trchounian

Yerevan State University, Department of Biochemistry, Microbiology and Biotechnology, Armenia
Armen Trchounian <Trchounian@ysu.am>

Plant origin secondary metabolites have served as antioxidants in medicines against various diseases. They have anticancer, anti-inflammatory and anti-aging properties and may reduce oxidative stress through a number of different mechanisms. In addition they can be used as effective natural additives in cosmetics.
The purpose of this research was to test biological activity of alcohol extracts obtained from the leaves of *Morus alba*, *Primula veris* L., *Malva sylvestris* L. and *Bryonia alba* L. roots which are used in Armenian folk medicine and have been included in some cosmetic formulations, as antioxidants. Plant material was harvested from Kotayk region (Armenia, 1700-1800 m above sea level). Antioxidant activity of extracts was measured through DPPH-assay (2,2-diphenyl-1-picrylhydrazyl), TBARs-assay (thiobarbituric acid reactive substances), metal-chelating and tyrosinase inhibitory ability determination. The concentration of total phenolics was determined using Folin–Ciocalteu reagent and external calibration with GA (gallic acid).
Our investigations have shown that *M. sylvestris* had the highest free radical reducing ability (IC_{50}=7.44 μg/ml), while IC_{50} value of *P. veris*, *M. alba* and *B. alba* were 87.5, 259.6 and 470 μg/ml, respectively. Fe^{2+} and Cu^{2+} ions could support the formation of free radicals *via* Fenton reaction. So, plant extracts ability of metal ion chelating is of great value. Our studies showed that *P. veris* had the highest metal-chelating power (56.0%), but *M. alba* and *M. sylvestris* had the similar activity, their percentage of metal ion inhibition was 29.9% and 29.6 %, respectively. *B. alba* had the lowest activity (5.6%). Plant extracts tyrosinase inhibitory activity is of special interest in cosmetic industry due to its skin lightening ability. *M. sylvestris*, *P. veris* and *B. alba* possessed almost the same ability to inhibit tyrosinase with 56.4, 58.2 and 58.6% inhibition, respectively. And only *M. alba* inhibited the 38.25% of tyrosinase in stock solution. In order to minimize the quantity of applicable laboratory animals, only one extract with the highest antiradical activity (*M. sylvestris*) had been chosen for TBARs assay. Lipid peroxidation was significantly inhibited in the presence of *M. sylvestris* extract when compared to the control ($P<0.05$). The TBARs assay indicated that AI% was 89.2% in comparison to the same concentration of positive control – α-tocopherol, where this parameter was 91.1%.
Total phenolic content of *B. alba* extract was 139.8, *M. sylvestris* extract – 152.81, *P. veris* extract – 308 and *M. alba* extract – 343.92 μg/ml GAE.
Thus, obtained data indicate that plants extracts investigated could be used as the positive sources of substances with antioxidant activity.