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F/8 CHANGES IN THE COLOCALIZATION BETWEEN Kv1.3 AND LIPID RAFTS IN RESPONSE TO ALTERATIONS IN STEROL CONTENT OF THE CELL MEMBRANE

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Keywords: membrane sterols, Kv1.3, lipid rafts, quantitative image analysis

Aims: The preferential lipid raft localization has been demonstrated in Kv1.3 voltage-gated potassium channels, which may modify its structural and functional properties. Our previous studies demonstrated that treatment with the major lipid raft constituent cholesterol exerts remarkable effects on electrophysiological parameters of Kv1.3. However, the exact molecular mechanisms of these alterations are unknown.

Methods: In our experiments, we analyzed the effects of cholesterol and 7-dehydrocholesterol (7DHC) on the lipid raft localization of Kv1.3. For microscopic analysis of the colocalization of the channels and lipid rafts, HEK-293 cells were transiently transfected with plasmids encoding Kv1.3. After loading of the cells with cholesterol or 7DHC, channels and rafts were visualized by confocal or stimulated emission depletion (STED) microscopy. During image analysis, the Pearson correlation coefficients between channel and raft marker signals were determined.

Results: First, we tested our method by analyzing the colocalization between two known raft markers, cholera toxin-B subunit and GFP-GPI. As expected, highly positive values of the correlation coefficient were found. Coefficients of similar magnitudes were determined between intensities of the raft marker and the antibodies against ion channels, demonstrating the preferential raft localization of Kv1.3. Furthermore, the extent of colocalization significantly increased in response to treatments with cholesterol and 7DHC. Our results were confirmed with STED microscopy providing much better (46-60 nm) resolution. **Conclusions:** In conclusion, changes in the distribution of Kv1.3 between different membrane microdomains might at least partially mediate the effects of sterols on these channels.

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F/9 VASOREGULATORY EFFECTS OF THE 18:2 LYSOPHOSPHATIDIC ACID (LPA) DEPENDS ON AGE

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Keywords: vasoconstriction, vasorelaxation, LPA, age, wire myography

Aims: Our research group has recently reported that LPA1 receptor activation in the endothelium induces vasorelaxation, while in the vascular smooth muscle (VSM) it evokes cyclooxygenase1 (COX1) activation with consequential thromboxane A2 (TXA2) release, which elicits vasoconstriction. During platelet-activation mainly 18:2 LPA is liberated. Since vascular diseases are more common in the elderly, in this study we aimed to observe the age-related changes of the 18:2 LPA induced vasoconstriction and -relaxation and to determine other

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Keywords: FXIII, fibrinogen, coagulation, thrombosis, interaction

Aims: Coagulation factor XIII (FXIII) consists of two A and two B subunits, its active form stabilizes the clot by crosslinking fibrin chains. Fibrinogen is made of three pairs of chains ($A\alpha$, $B\beta$, γ). Plasma fibrinogen can be separated by ion exchange chromatography into 3 fractions: Peak1 containing $\gamma A/\gamma A$ chains (85%), Peak2 with $\gamma A/\gamma'$ (15%) chains and γ'/γ' (0.5%) fraction. The plasma FXIII circulates in the blood bound to fibrinogen. By characterizing this interaction, we may be able to synthesize a peptide that binds to FXIII and decreases its activity and consequently thrombus formation.

Methods: Our aim was to characterize the binding of FXIII to the different fibrinogen isoforms by surface plasmon resonance technique (SPR) and isothermal titration calorimetry (ITC). We used Biacore systems for the SPR measurements and we immobilized FXIII-B and Peak1 fibrinogen as the ligand. As analyte, we used FXIII-B, Peak1, Peak2, and a 9-amino-acid long γA ($\gamma A9$) peptide, which we made by peptide synthesis. We studied the interaction between FXIII and $\gamma A9$ on a microcalorimeter (Microcal ITC 200).

Results: Peak1 and Peak2 fibrinogen bind to FXIII-B with almost the same affinity (K_d in the range of 10^{-6}) and the $\gamma A9$ binds to FXIII-B with a two magnitudes higher affinity (K_d in the range of 10^{-8}).

Conclusions: In conclusion, we can confirm that the FXIII-B subunit is associated with fibrinogen. The binding is a complex mechanism involving both γA and γ' chains.

M/5 THE MEASUREMENT OF THE DIPOLE POTENTIAL WITH A NOVEL HIGH-THROUGHPUT FLOW CYTOMETRIC METHOD

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Keywords: dipole potential, hypercholesterolemia, polyunsaturated fatty ac

Aims: The least-known component of membrane potentials, the dipole potential (DP) originates from the preferential alignment of molecular dipoles at the membrane-water interface, generating a large positive (~ 300 mV) potential creating an immensely strong intramembrane electric field. Consistently, DP influences a multitude of membrane-coupled events, including ion channel gating, ErbB activation, etc. The magnitude of DP is mainly determined by the lipid composition of cell membrane. Since it is entirely located within the membrane, it is difficult to measure in living cells, however, we developed a flow cytometric technique to determine DP. Our aim was to examine effects of changes in the composition of cell membrane in disease models and human samples.

Methods: As models of hypercholesterolemia and Smith-Lemli-Opitz-syndrome, we treated THP1, JY cells or human PBMC with cholesterol or 7-dehydrocholesterol (7DHC), respectively. To test the beneficial effects of Mediterranean diet, we treated cells with polyunsaturated fatty acids. Then we measured the magnitude of DP with our novel emission ratiometric approach using flow cytometry.

Results: Loading of cells with cholesterol or 7DHC resulted in dose-dependent increases in DP. As demonstrating

the importance of diet, ω -3 fatty acid decreased, while ω -6 or saturated fatty acid increased the magnitude of DP. In our ongoing studies, we are determining alterations in the DP in samples from hypercholesterolemic and hypertriglyceridemic patients compared to control individuals. **Conclusions:** In conclusion, we determined the effects of changes in membrane composition on DP in in vitro disease models with our novel technique.

M/6 DRUG CANDIDATE BGP-15 IMPROVES CARDIAC FUNCTION BY ACTIVATING THE PROTEIN KINASE G PATHWAY

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Keywords: Heart failure, Dyslipidemia, BGP-15, Echocardiography, cGMP-PKG

Aims: Pharmacological treatment of heart failure represents a major unmet need. Recent evidence suggests that hydroximic-acid derivative small molecule BGP-15 improves muscle function and strength, restores cardiac function, and reduces arrhythmic episodes in animal models of muscular dystrophy, heart failure or diabetic cardiomyopathy. Our aim in this present study was to evaluate the effects of BGP-15 in a rabbit model of atherosclerotic cardiovascular disease.

Methods: New-Zealand male rabbits were divided into 3 groups: (I) healthy control (normal chow); (II) dyslipidemic (DD) group (atherogenic chow); (III) atherogenic chow+BGP-15-treatment (DD+BGP-15 group, 10 mg/kg, p.o.), for 16 weeks. Serum parameters were determined at the endpoint of the study, and echocardiography was carried out to estimate cardiac function. Western Blot and ELISA assays were carried out to determine myocardial cGMP levels and VASP protein phosphorylation to determine the activity of the cGMP-PKG signaling pathway.

Results: Serum parameters were unaffected by BGP-15-treatment. The drug candidate restored diastolic function (E and A wave, DecT, e'/a', E/e', IVRT) and improved Tei-index compared to untreated DD rabbits. Myocardial cGMP levels, along with the phosphorylation of VASP protein was significantly elevated in the left ventricle samples of BGP-15-treated rabbits in comparison to the DD animals.

Conclusions: Even though BGP-15 treatment did not affect serum parameters, it considerably enhanced diastolic function, partly by modulating the cGMP-PKG pathway. As BGP-15 has excellent safety profile, it may offer a new therapeutic approach in atherosclerotic cardiovascular disease.

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M/7 IMPACT OF PER OS ANTIBIOTIC TREATMENTS ON THE GASTROINTESTINAL COLONIZATION WITH A MULTIRESTANT KLEBSIELLA PNEUMONIAE STRAIN

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Keywords: AMR, microbiota, gene transfer, colonization, in vivo

Aims: Colonization resistance refers to the ability of microbiota to prevent expansion and persistence of exogenously acquired bacterial species. Antibiotic therapy can damage this ability. Our goal was to determine

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With questions or suggestions, please contact the Editor at ssomkuti@abington-repromed.com.

*Thank you,
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