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41ST ANNUAL SCIENTIFIC CONFERENCE OF THE ROMANIAN SOCIETY FOR CELL BIOLOGY

WITH INTERNATIONAL PARTICIPATION UNDER THE AUSPICES OF GEORGE EMIL PALADE UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE, AND TECHNOLOGY OF TARGU MURES

Targu Mures, Romania
11-13 December, 2024

BOOK OF ABSTRACTS

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41st Annual Scientific Conference of the Romanian Society For Cell Biology with International Participation under the auspices of George Emil Palade University of Medicine, Pharmacy, Science, and Technology of Targu Mures

Targu Mures, Romaniam December 11-13, 2024

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FOREWORD

Dear Colleagues,

I welcome you all to the 41st Annual Scientific Session of the Romanian Society for Cell Biology. I am pleased to present to you the Bulletin of our Society which contains the abstracts of the presentations held at the 2024 – Session of our Society.

This collection of abstracts brings together the work of the members of our Society who are trying to push the boundaries of our understanding of the biological world, exploring new frontiers and addressing critical challenges.

Today, the field of cell and molecular biology is going through an ample revolution. Novel technologies, hard work and interdisciplinary collaborations led to a better understanding of living systems. The abstracts within this volume capture the essence of this dynamic landscape, presenting research that spans a wide spectrum of biological sub-disciplines.

We are lucky to have this congress in the beautiful Tg. Mures, a city full of history, hardworking and serious people, a reputed University of Medicine that created the best framework for a successful scientific congress.

I extend my appreciation to the dedicated scientists whose work graces these pages and contribute to the advancement of biological knowledge. I encourage you to read the abstracts, capture the scientific content, learn novel data, explore new topics and potential collaborations and be active in the effort to shape the future of cell and molecular biology.

I hope that this book of abstracts inspires curiosity, fosters collaboration, and contributes to the ongoing dialogue that propels biological research forward.

A word of gratitude to professor George Palade whose talent, intuition, vision, and hard work, together with professors Albert Claude and Christian de Duve received the Nobel Prize in 1974 for introducing Cell Biology - a new field of science - in the world.

We extend our gratitude to Prof. Nicolae Simionescu, the founder of the Cell Biology discipline in Romania and of the Romanian Society for Cell Biology.

Our great appreciation to all the authors who have contributed to this book.

We look forward to the exciting discussions and collaborations that will emerge from this year's congress of the Romanian Society for Cell Biology.

Acad. Maya Simionescu

President of the Romanian Society for Cell Biology

PHD STUDENTS' POSTER SESSION 1

CARDIAC HYPERTROPHY IN ATHEROSCLEROTIC CARDIOVASCULAR DISEASE: MOLECULAR MECHANISMS AND THERAPEUTIC APPROACHES

Alexandra Vilcu, Ioana Karla Comarița, Alina Constantin, Nicoleta Alexandru-Moise, Miruna Nemecz, Gabriela Tanko, Adriana Georgescu

Institute of Cellular Biology and Pathology 'Nicolae Simionescu' of Romanian Academy, Bucharest, Romania

Background: Atherosclerosis, one of the most popular cardiovascular pathologies marked by blood vessel narrowing is often associated with other complications such as cardiac hypertrophy.

Aim: In this study, we followed the changes that appear at the structural, functional, and molecular levels following the treatment with extracellular vesicles (EVs) isolated from 2 different sources: subcutaneous adipose tissue stem cells (ADSCs) and bone marrow mesenchymal stem cells (BM-MSCs), with or without Smad2/3 siRNA transfection.

Methodology: The experiments were performed on Golden Syrian hamsters, followed for 4 months, divided into seven experimental groups: (1) Control (C), normal healthy animals; (2) HH generated by combining a special atherogenic diet with daily 8% NaCl gavage; (3) HH-EVs(ADSCs); (4) HH-EVs(BM-MSCs), (5) HH-EVs(ADSCs)+Smad2/3siRNA; (6) HH-EVs(BM-MSCs)+Smad2/3siRNA; (7) HH-Smad2/3siRNA. Treatments were administered by retro-orbital sinus or subcutaneous injection of 100µg/ml EVs transfected or not with 100 nM Smad2/3 siRNA or with Smad2/3 siRNA alone.

Results: At the end of the diet and/or treatment we noticed some improvements: (1) a marked decrease in the levels of plasmatic parameters, including TGF-β1 and Ang II; (2) structural and functional regeneration in the left ventricle; (3) a reduction in the expression of inflammatory markers involved in cardiac hypertrophy; and (4) a diminished protein expression profile of Smad2/3, ATF-2, and NF-kBp50/p65.

Conclusion: The EVs-based treatment, both with and without Smad2/3 siRNA transfection, resulted in a notable enhancement in left ventricular structure and function, accompanied by a reduction in inflammatory marker levels.

Acknowledgments: This research was supported by the CNCS-UEFISCDI grant from the Romanian Ministry of Education and Research (PN-III-P1-1.2-PCCDI-2017-0527/Contract 83PCCDI/2018) and the Romanian Academy.

NUCLEOLIN: A NEW MOLECULAR TARGET FOR SPECIFIC DRUG DELIVERY TO REDUCE CARDIOVASCULAR INFLAMMATION

Delia Boteanu, Maria Anghelache, Manuela Călin

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Background: Nucleolin (NCL) is a multifunctional protein crucial in maintaining cellular function. Elevated expression of NCL has been linked to inflammation and has recently been associated with cardiovascular disease onset. We hypothesize that NCL can serve as a suitable target for dexamethasone (Dexa) delivery to activated endothelium.

Aim: To develop Dexa-loaded liposomes (LP-Dexa) coated with an affinity peptide for NCL (F3/LP-Dexa) to target activated endothelial cells (EC) and reduce their inflammatory status.

Methodology: LP-Dexa were obtained using the lipid film hydration followed by the extrusion method and functionalized with F3 peptide via thiol-maleimide linkage to the LP/Dexa surface. The internalization of Dexa-loaded liposomes by EC was evaluated at different intervals using immunofluorescence and flow cytometry. NCL protein and gene expression were analyzed after activation of EC and treatment with nanoparticles using immunofluorescence, flow cytometry, and real-time PCR. The anti-inflammatory effect of F3/LP-Dexa was evaluated) using Western blot probed with monocyte chemoattractant protein-1 (MCP-1) antibody.

Results: The NCL expression was 2-fold increased on the surface of TNF-α-activated bEnd.3 cells compared with non-activated cells. bEnd.3 cells efficiently internalized liposomes at 30 and 90 minutes of treatment, with F3/LP-Dexa being efficiently internalized at 90 minutes, compared to LP-Dexa. LP-Dexa and F3/LP-Dexa at lower concentrations of incorporated Dexa (1 µM; 2,5 µM; 5 µM; 10 µM) did not induce cytotoxic effects, although a concentration of 20 µM significantly reduced the cellular viability. Gene expression of NCL was significantly increased in activated cells compared to control cells. The treatment with F3/LP-Dexa significantly reduced the expression of NCL mRNA in activated cells compared to Dexa-treated cells. Moreover, a decrease in MCP-1 protein expression was observed in F3/LP-Dexa treated cells compared to non-treated cells.

Conclusion: NCL exposed on the surface of endothelial cells can be used as a shuttle molecule for intracellular delivery of drug nanocarriers and could contribute to vascular inflammation reduction.

Acknowledgement: This work was supported by Romania's National Recovery and Resilience Plan, PNRR-III-C9-2022-I8, CF 93/15.11.2022, Financing Contract no. 760063/23.05.2023.

ANTI-INFLAMMATORY N2 NEUTROPHILS REPROGRAM MACROPHAGES TO ADOPT A PRO-HEALING PHENOTYPE WITH ENHANCED EFFEROCYTOSIS CAPACITY

Andreea C. Mihaila, Monica Tucureanu, Letitia Ciortan, Maya Simionescu, Elena Butoi

Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania

Introduction: After myocardial infarction (MI), neutrophils rapidly infiltrate the heart, where they are temporally polarized into pro-inflammatory (N1) and anti-inflammatory (N2) sub-populations. This neutrophil migration is followed by the recruitment of macrophages, which are thought to undergo phenotypic shifts from pro-inflammatory to pro-healing macrophages, facilitating inflammation resolution. We hypothesize that N2 neutrophils may reprogram macrophages toward a pro-healing phenotype, enhancing their efferocytosis capacity and performed experiments investigating the effect of factors released by N2 neutrophils on macrophages.

Materials and Methods: To investigate this, human neutrophils isolated from healthy donors were polarized into N1 and N2 sub-populations, and their secretomes were added to human macrophages derived from THP-1 monocytes. The effects of neutrophil-derived factors on macrophages were assessed using qPCR for gene expression, ELISA for soluble proteins, Western blotting for cellular proteins, immunofluorescence for efferocytic receptors, and efferocytosis assays.

Results: The results showed that factors released by N2 neutrophils modulate macrophage phenotype and functionality by inducing: i) increased gene and protein expression of anti-inflammatory molecules (TGF-β, IL-10 and CD206) as well as of key nuclear factors associated with reparative macrophages (Nur77, KLF4 and PPARγ); ii) elevated expression of efferocytosis receptors (MerTK, αv/β5 integrins, CD36, CX3CR1) and bridge molecules (Gas6 and Mfge8); and iii) improved efferocytosis capacity.

Conclusion: These data highlight the role of factors released by anti-inflammatory N2 neutrophils in inducing a reparative phenotype in macrophages. Specifically, N2 neutrophils promote the expression of anti-inflammatory molecules and nuclear factors associated with reparative macrophages. Additionally, they upregulate molecules involved in efferocytosis, such as MerTK, Mfge8, and Gas6. Together, our findings reveal a novel and significant function of the anti-inflammatory N2 neutrophil subtype: amplifying macrophages' efferocytosis capacity, a crucial mechanism for resolving inflammation.

Acknowledgement: This work was supported by Romania's National Recovery and Resilience Plan, NextGenerationEU, PNRR-III-C9-2022-I8-186/24.11.2022, FibroThera project, Financing Contract no. 760062/23.05.2023, and by the Romanian Academy.

Keywords: pro-healing macrophages; neutrophil secretome; MerTK; efferocytosis; bridge molecules

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DEVELOPMENT OF BIOMIMETIC NANOPARTICLES FOR DELIVERY OF ANTI-FIBROTIC AGENTS TO CARDIAC MYOFIBROBLASTS

Ruxandra Anton, Maria Anghelache, Geanina Voicu, Delia Boteanu, Florentina Safciuc, Rostyslav Bilyy, Manuela Călin

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Background: Cardiac fibrosis is characterized by an excessive accumulation of fibrous connective tissue in the heart. The main effectors of cardiac fibrosis are myofibroblasts. Biomimetic nanoparticles (Bio-NP) are emerging as innovative approaches in targeted drug delivery, exploiting the inherited biological functions of origin cells for enhanced specificity and therapeutic efficacy. We hypothesize that a targeted approach based on Bio-NP effectively treats cardiac fibrosis by reducing fibrosis, improving cardiac function, and mitigating inflammation by modulating the fibrotic microenvironment.

Aim: To develop neutrophil membranes-covered lipid nanoparticles that can accumulate in the injured heart and deliver locally anti-fibrotic agents.

Methodology: The oil phase containing L-α-Phosphatidylcholine, soybean oil, and an anti-fibrotic (AF) compound (i.e. 17-AAG, an HSP90 inhibitor) was mixed with an aqueous phase containing glycerol and water and nanoemulsions (LN/AF) were obtained through sonication. These LN/AF were coated with polyethyleneimine (PEI), and covered with membranes isolated from neutrophils to obtain Bio-LN/AF. The nanoparticles were characterized for size, Zeta potential, and AF entrapment efficiency. The presence of neutrophil membrane proteins on the surface of Bio-LN/AF was investigated by Western blot and flow cytometry. The binding and uptake of Bio-LN/AF by murine cardiac fibroblasts were investigated at different intervals using fluorescently labeled Bio-LN/AF by flow cytometry and fluorescent microscopy.

Results: Western blot and flow cytometry analyses confirmed the presence of specific neutrophil receptors on the nanoparticle surface. The stability of Bio-LN/AF was assessed over one month, revealing a maintained hydrodynamic diameter (~160 nm) and stable surface potential (23 mV). The activated cardiac fibroblasts took up the Bio-LN/AF but at a lower rate when compared to LN/AF.

Conclusion and perspective: The neutrophil membrane-covered nanoparticles need further optimizations to be validated as vectors for improving targeted drug delivery and therapeutic outcomes in cardiac fibrosis.

Acknowledgement: This work was supported by Romania's National Recovery and Resilience Plan, PNRR-III-C9-2022-I8, CF 93/15.11.2022, Financing Contract no. 760063/23.05.2023.

THE ANTIMICROBIAL POTENTIAL OF THE *ARTHROSPIRA PLATENSIS* ETHANOLIC EXTRACT

Isabella Stoian[1,2,3], Daniela Pușcașiu[1,2], Roxana Popescu[1,2], Mihai Mitulețu[1,2], Teodor Cerbulescu[1,2], Ion Valeriu Caraba[4], Marioara Nicoleta Caraba[1,2]

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4. Faculty of Bioengineering of Animal Resources, University of Life Sciences "King Mihai I" from Timisoara, Calea Aradului, 119, Timisoara, 300645, Romania.

Introduction: *A. platensis* extracts due to their complex chemical compositions (proteins, carbohydrates, essential fatty acids, vitamins, minerals, pigments, carotenoids, chlorophyll a, phycocyanin, phycoerythrin, bio-active secondary metabolites, including phenolic compounds) represent an important source in the industry pharmaceutical. The antimicrobial potential of spirulina extracts has increased the interest of groups of researchers for extensive, extended studies in the area of bacteria showing antibiotic resistance, considering spirulina a safe and often more effective alternative compared to synthetic antimicrobial agents. Spirulina is considered an important natural preservative, but also an antimicrobial agent for pathogenic bacteria and fungi, including drug-resistant microorganisms.

Material and Method: The ethanolic extract of A. platensis was tested in 5 different concentrations, using the diffusion method, measuring the values of inhibition zones obtained when the extract was applied. Antimicrobial activity was studied on standardized bacterial strains, Gram+ bacteria: ***Staphilococcus aureus*** (ATCC 25923), ***Streptococcus pyogenes*** (ATCC 19615) and Gram- bacteria: ***Shigella flexneri*** (ATCC 12022), ***Pseudomonas aeruginosa*** (ATCC 10145), ***Escherichia coli*** (ATCC 25922), ***Salmonella typhimurium*** (ATCC 14028), ***Haemophilus influenzae*** type B (ATCC 10211).

Results and Conslusions: The Gram+ and Gram- bacterial strains taken in the study showed a different response to the ethanolic extract concentrations tested, there being variations from the absence of antibacterial effects for most of the strains to an obvious antibacterial effect. The ethanolic extract of spirulina tested in this study resulted in insignificant growth inhibition for the two Gram+ bacterial strains. In the case of Gram bacterial strains, the effects of the five concentrations of the ethanolic extract varied significantly depending on the strain and the applied concentration. The ethanolic extract of spirulina at the first concentrations tested was antibacterial for the strains of ***S. typhimurium*** and ***E. coli,*** the decrease in the concentration of the extract led to the decrease of the antibacterial effect.

USE OF MMP-9 AND D-DIMER LEVELS AS A MARKER FOR IDENTIFYING ABDOMINAL AORTIC ANEURYSMS IN PATIENTS WITH SIGNIFICANT CORONARY ATHEROSCLEROSIS

Diduta Alina Brie[1,2], Ioana Muntean[1,2], Isabella Stoian[1,2] , Teodor Cerbulescu[1,2] , Daniela Pușcașiu[1,2], Daniel Miron Brie[3]

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3. Department of Interventional Cardiology, Cardiovascular Disease Institute Timisoara, Gheorghe Adam St., No.13A, postal code 300310 Timisoara, Romania.

Aim and objectives: Our study aims to identify the link between the levels of specific biomarkers (MMP-9 and D-Dimers) and the occurrence of AAA in high-risk groups of patients diagnosed with significant atherosclerotic coronary disease. Establishing a correlation between these markers and AAA could improve current screening **Methods:**

Materials and Methods: We have screened patients with angina pectoris and indication for coronary angiography for diagnosis of AAA. After performing coronary angiography, patients diagnosed with significant atherosclerotic coronary disease underwent peripheral arteriography to identify aortic aneurysms. We have three categories of patients- patients with significant CAD and AAA (group A), patients with substantial CAD without AAA (group B), and patients with exertional angina without significant CAD and AAA (group C-control group). Fifty patients from each category were selected, and we measured their MMP-9 and D-dimer serum levels.

Results: The mean MMP-9 value was increased in group A(96.5 ± 16.9 ng/mL) but not in group B (48.7 ± 10.5 ng/mL)- p=0.017, or group C (23.4 ± 8.4 ng/mL)- (p=0.001)- Figure 1. The D-Dimer level was also increased in group A (1.2 ± 0.5 mg/L) but not in group B(0.62 ± 0.3 mg/L), C (0.28 ± 0.14 mg/L) p<0.001- Figure 2. Our data showed no correlation between the increased MMP-9 level in group A and aneurysm diameter (Spearman correlation rho=0.203) and between D-Dimer level and AAA diameter (rho=0.273). ROU curve for MMP-9- AUC value 0,6293 (95%CI, 0,5905-0,698)-Figure 3 and ROU curve for D-dimer- AUC value 0,7138 (95% CI 0,6410-0,7628).

Conclusions: In our study, patients with significant coronary disease and abdominal aortic aneurysm had statistically significantly increased values of MMP-9 and D-Dimers compared to those who only have coronary disease without abdominal aneurysm, or to the control group (without coronary disease, without aneurysm). Increased levels of MMP-9 and D-Dimer in patients with significant coronary artery disease and AAA did not correlate with AAA diameter.

APLICATION OF SPECULAR MICROSCOPY IN MANAGEMENT OF CORNEAL DISEASE

Teodor Cerbulescu[1,2,3] , Marioara Nicoleta Caraba[1,2], Isabella Stoian[1,2], Diduta Alina Brie[1,2], Roxana Popescu[1,2]

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Introduction: Specular microscopy is a non-invasive imaging technique that allows for the detailed evaluation of the corneal endothelium, which plays a critical role in maintaining corneal clarity and fluid balance.

Material and Method: This technique is particularly useful in the diagnosis, monitoring, and management of various corneal diseases. Here's an overview of its applications in managing corneal disease: Diagnosis and Monitoring of Corneal Endothelial Disorders, Preoperative and Postoperative Assessment in Corneal Surgery, Management of Contact Lens-Induced Endothelial Stress, Screening and Management in Glaucoma and Ocular Hypertension, Evaluation of Endothelial Health in Diabetic Patients, Research and Development in Corneal Therapies, Corneal Edema Assessment. Advantages of Specular Microscopy in Corneal Disease Management: Non-Invasive and Quick: It provides high-resolution images of the endothelium without invasive procedures; Quantitative Data: Enables tracking of cell density, morphology, and variation, allowing for precise monitoring; Early Detection of Pathologies: Identifies subtle changes in endothelial cells before clinical symptoms develop.

Results and Conclusion: In summary, specular microscopy is an essential tool in managing corneal diseases, offering detailed insights into endothelial health that inform diagnosis, surgical planning, and postoperative care. Its role is invaluable in maintaining and restoring vision by enabling targeted, informed interventions in corneal care.

PRESENTATION SESSION 1

DRUG DELIVERY SYSTEM TO SLOW THE GROWTH OF ABDOMINAL AORTIC ANEURYSMS; A TRANSLATIONAL JOURNEY

Dan Simionescu, Agneta Simionescu

Department of Bioengineering, Clemson University, Clemson, SC, USA

Abdominal aortic aneurysms (AAA), characterized by focal progressive dilatation of the aorta, affect millions of patients globally and thus constitute a considerable health concern. AAA is caused by chronic inflammation, proteolytic degradation, and aortic wall weakening that can fatally burst if not detected or treated on time. The current treatment paradigm includes active surveillance for small-to-medium AAAs (4-5 cm in diameter) and surgery for patients with AAA diameters >5.5 cm. Currently, there is no drug therapy or other non-surgical treatment option for patients with small-to-medium AAAs to minimize or prevent AAA growth. Our working hypothesis was that local delivery of a matrix binding agent capable of stabilizing aortic elastin and collagen and reducing their susceptibility to proteolytic degradation could delay the growth of AAA and significantly extend the time to surgery.

The scientific premise of this project was based on the discovery that 1,2,3,4,6-Pentagalloyl glucose (PGG), a naturally occurring polyphenol, is a potential AAA treatment due to its ability to bind strongly to elastin and collagen and protect the extracellular matrix from pathologic degradation. This lecture presents the 20-plus-year “bench to bedside” translational journey of this idea. “Benchwork” included proteolytic studies, molecular modeling, and peptide mapping techniques to understand molecular interactions of PGG with normal and pathologic aortic wall proteins. We also tested the diffusion characteristics of PGG throughout aortic tissue and through intra-luminal thrombus (ILT), a typical occurrence in developing AAA. A novel patented double-balloon delivery system was designed and manufactured to localize the administration of PGG to the aneurysmal sac using a shape-fitting “weeping balloon” device. We then showed that PGG preserves elastin integrity and significantly inhibits AAA progression when delivered to the aneurysmal aorta in an AAA rodent model, in an AAA swine model and in a first-in-human-clinical trial in 20 patients. Throughout our studies, PGG was deemed locally and systemically safe and non-toxic, passing all required biocompatibility tests, including major organ toxicity.

In summary, a deep understanding of chemistry and mechanisms of action, laborious benchtop experimentation, arduous safety testing, extensive animal studies and a pilot study with human patients suggest that PGG may have the potential to be a safe and effective non-surgical treatment of small-to-medium-sized AAA.

DYNAMIC MODEL TO MIMIC EARLY CARDIAC FIBROSIS IN DIABETES

Agneta Simionescu

Department of Bioengineering, Clemson University, Clemson, South Carolina, USA

Diabetes Mellitus affects millions of people worldwide, with the number of patients increasing dramatically every year. All cells, tissues and organs in the body are affected by diabetes, including the heart. Maladaptive responses to the hyperglycemic and hyperlipidemic environment alter the microstructure of the heart, resulting in organ-wide perivascular as well and interstitial fibrosis. Currently there is no treatment for targeted mitigation of the impact of diabetes on the heart. Existing treatments include changes in diet and lifestyle and bypass surgery for treatment of myocardial infarction and heart transplantation for treatment of advanced cardiomyopathy.

The aim of this study was to develop a biomimetic model for cardiac pathologies, based on tissue engineering principles. A decellularized matrix derived from porcine left ventricle served as a scaffold. This was seeded with cardiac cells and mounted in a bioreactor that provided physiological or pathological conditions. The model allowed us to focus on the normal and modified biochemical reactions in the diabetic heart. Cardiac fibroblasts cultured in 2D and 3D (as spheroids) provided baseline data related to collagen synthesis and degradation. The co-culture of fibroblasts and cardiomyocytes seeded on scaffolds completed the model. The analysis of TGF-β, collagen type 1, and MMP 2&9 showed changes in the cellular and extracellular components of the heart under high glucose conditions. To further elucidate the intercellular communication under pathological conditions, exosomes were isolated from the cell culture media, and miRNA 21 was found to be involved in cell communication under diabetic conditions.

This research will advance the understanding of the mechanisms responsible for cell responses to altered cues in diabetes and contribute to the cardiac tissue engineering field.

DEVELOPMENT OF CORTICAL ORGANOIDS TO STUDY MITOCHONDRIAL DISORDERS

Denisa Hathazi

University of Cambridge, Department of Clinical Neurosciences, John Van Geest Cambridge Centre for Brain Repair, Cambridge United Kingdom

Mitochondrial diseases encompass a heterogeneous group of disorders that frequently affect the brain, often resulting in progressive, disabling, or fatal outcomes, with limited effective therapeutic options. Among these, MELAS syndrome—also caused by the m.3243A>G mtDNA mutation—is a severe form of mitochondrial disease characterized by epilepsy, stroke-like episodes, and cognitive impairment. In vitro modeling of such complex disorders remains challenging due to factors like fluctuating heteroplasmy (the ratio of wild-type to mutant mitochondria), variations in mitochondrial copy number, and nuclear-genomic interactions.

To address these challenges, we developed cortical organoids from patient-derived induced pluripotent stem cells (iPSCs) harboring the m.3243A>G mutation. These organoids were generated from iPSCs with varying heteroplasmy levels from the same patient and were cultured up to 200 days. The models demonstrated stable heteroplasmy, mitochondrial dysfunction, and the presence of diverse cellular populations, including mature neurons, glia, astroglia, and various progenitor cells. We observed that impaired bioenergetics disrupted neuronal structure and function, leading to a significant loss of deep-layer neurons, particularly those in layer V. Functional analyses using multielectrode array (MEA) on day-200 organoids revealed pronounced hypersynchrony in the neuronal network, especially under mitochondrial stress conditions.

These models represent a valuable tool for developing and testing novel therapies aimed at ameliorating the neurological phenotypes associated with mitochondrial diseases like MELAS.

MORE THAN HIRED KILLERS: NON-APOPTOTIC FUNCTION(S) OF APOPTOSIS AND CASPASE-3 IN CANCER CELL MOTILITY

Gabriel Ichim

Cancer Research Center of Lyon (CRCL) INSERM 1052, CNRS 5286, Lyon, France. Cancer Cell Death Laboratory, part of LabEx DEVweCAN, Université de Lyon, France

In cancer, it is unequivocally established that apoptosis is an efficient roadblock by efficiently removing transformed cells. However, our team and others showed that oncogenesis re-appropriates apoptosis and its effectors (mainly caspases) to fuel certain of its hallmarks such as cancer cell invasion. Indeed, our team showed recently that low-level caspase activation in the so-called failed apoptotic cells is compatible with survival and moreover it promotes melanoma aggressiveness. Using a sensitive caspase activation reporter, we isolated and thoroughly characterized melanoma cancer cells surviving the induction of apoptosis. Importantly, our results suggest these cells have a particular transcriptomic signature associated with cellular motility. In line with this, cells surviving apoptosis have a gain in aggressiveness: they have an increased migration and invasion potential both in vitro and in vivo. We further demonstrate that failed apoptosis-associated gain in invasiveness is regulated by the JNK pathway while its transcriptomic signature can discriminate primary from metastatic human melanoma tumors. In a complementary study we tested whether caspase-3 has protease-independent function in melanoma cell motility. We now have preliminary data that procaspase-3 but not procaspase-7 is required for melanoma cell migration and invasion, since its down regulation impairs chemotaxis, collective migration and invasion. In addition, caspase-3 IP-MS proteomic analysis revealed that most putative interacting proteins are associated with the actin cytoskeleton. To take this study further, we now perform several proteomic assays such as BioID to establish how and where exactly caspase-3 interferes with melanoma cell motility.

ARTEMISININS RESCUE THE EXPRESSION OF KEY PROTEINS OF INHIBITORY SYNAPSES IN AN ALZHEIMER'S DISEASE MODEL

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Alzheimer's disease (AD), especially its sporadic, late onset form is the most prevalent form of dementia, affecting more than 45 million people worldwide. This disease is characterized by progressive and irreversible degeneration of the brain, related to disruptions in memory, cognition, and personality, with huge implications for present and future societies. Important neuropathological hallmarks of AD, common for both familial and sporadic forms of the disease, include extracellular deposits of β amyloid protein (Aβ) in plaques and intracellular deposits of hyper-phosphorylated tau protein as fibrillary tangles in different brain regions. Besides mitochondria failure and inflammation, the loss of synapses is one other major hallmark of AD. Patients as well as different mouse models carrying mutations in genes that cause early-onset familial AD such as the amyloid-protein precursor (APP) gene, result in accelerated production of the proteolytic cleavage product Aβ and exhibit impairment of hippocampal synaptic plasticity long before the onset of clinical symptoms. It is known that, in addition to excitatory synapses, inhibitory GABA (g-aminobutyric acid)-ergic neurotransmission is also effected in the AD brain. Inhibitory neurotransmission in the central nervous system is mediated by pentameric GABAA- and Glycine receptors (GlyRs) that are formed by the co-assembly of different subunits. GlyRs and a subset of GABAA receptors are anchored at postsynaptic membrane specializations by the multifunctional scaffolding protein gephyrin. Because AD is recognized as a multifactorial disease, and considering the only very limited clinical effects of most single-target therapies directed specifically against Aβ, it seems to be essential for future therapeutic approaches to target several pathogenic factors of the disease, in particular, the pathology of synapses. We used the AD mouse model APP-PS1 to study the expression of key proteins of inhibitory synapses in the hippocampus in conditions of increased amyloidosis. Moreover, we used this model to test the hypothesis that the anti-malaria medicine Artemisinin is a multi-target drug that might affect synapse pathology in AD. Artemisinin and its derivatives (collectively termed as artemisinins) are sesquiterpene lactones derived from the plant sweet wormwood (*Artemisia annua*), which has been applied in traditional Chinese medicine to treat "fever". Currently, artemisinins are first-line drugs in the treatment of malaria caused by Plasmodium parasites. For the isolation and discovery of artemisinin, the active compound of the plant, as an anti-malarial drug, the Chinese scientist Tu Youyou received the Nobel Prize in Physiology or Medicine in 2015. We found that at 12 months of age, the g2 subunit of the GABAA receptor as well as the scaffold protein gephyrin and its phosphorylation was significantly reduced in the hippocampus of APP-PS1 mice. In addition, specifically the GlyRa3 subunit showed also significantly lower protein expression level in comparison to wild type mice. The treatment of the APP-PS1 mice with 10 mg/kg or 100 mg/kg Artemisinins for three months rescued the expression of these three key proteins to about wild type levels. Importantly, at the same time the level of Ab, APP C-terminal fragments (CTFs), and of the plaque load in hippocampus and cortex were reduced significantly by this treatment. The identification of alterations in brain structure and function in early, pre-symptomatic stages is thought to be of crucial importance for protective interventions. Thus, we analyzed also APP-PS1 mice at 3 months of age, when amyloid plaques start to develop, and found that not only the level of gephyrin but also the phosphorylation of gephyrin and tau proteins are increased already at this early stage of disease. Consistently, elevated CDK5 and p35 protein levels, both involved in tau phosphorylation, were detected. Moreover, we could show that CDK5 and p35 are functionally involved in the increased phosphorylation of gephyrin at Ser270, resulting in significant increase of gephyrin- and g2- protein density at postsynaptic sites. Importantly, artemisinin treatment for three weeks of APP-PS1 mice at these younger stages (3 months) also increased CDK5 and p35 gephyrin phosphorylation at Ser270, thus effecting postsynaptic protein density of GABAA receptors.

In conclusion, our data strongly support the hypothesis that artemisinins target key proteins of inhibitory synapses and might restore synapse functions in AD synapse pathology.

PRESENTATION SESSION 2

STATINS: A DOUBLE-EDGED SWORD IN FUTURE CANCER TREATMENTS – RISKS AND REWARDS

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Statins possess antitumor activity at concentrations 100- to 500-fold higher than those needed for reaching cholesterol lowering activity. As lipophilic statins can accumulate passively intracellularly, they are superior to hydrophilic statins in terms of antitumor effects. Our data also proved that simvastatin (SIM) -a lipophilic statin exerted strong antitumor action on B16.F10 murine melanoma cells via strong suppression of the intracellular expression of the transcription factor HIF-1 under hypoxia as well as under normoxia (3,4). Nevertheless, it seemed that under normoxia SIM directed lipid metabolism toward favoring melanoma cell survival. To counteract this limitation of SIM-based treatments, combination anticancer therapies have been explored. Thus, our data have demonstrated the potential of tumor-associated macrophage-targeted liposomal SIM to enhance antiangiogenic as well as cytotoxic targeted treatments when they were co-administered in cancer models *in vivo*.

This work was supported by L'Oréal - UNESCO "For Women in Science" [Fellowship Programme no. 914, 2020] and the UEFISCDI grants [code PN II – RU 387/2010, No. 145/2010; PN-II-RU-TE-2014-4-1191; No. 235/2015; PN-III-P4-ID-PCE-2016-0342, (contract 91/2017) PN-III-P2-2_1-PED-2021-0411, No. 659PED, 2022].

NUTRITIONAL STATUS IN A COHORT OF ROMANIAN PAEDIATRIC PATIENTS WITH PHENYLKETONURIA

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Introduction: Phenylketonuria (PKU) is an autosomal recessive congenital disorder of L-phenylalanine metabolism caused by mutations in the phenylalanine hydroxylase (PAH) gene. PKU is a chronic condition that requires rigorous nutritional management. It involves restricted diets low in natural proteins supplemented with special protein substitutes. The nutritional status of PKU patients is particularly important and correlated with quality of life. The PKU diet, which is required to control phenylalanine (Phe) levels, often leads to deficiencies of essential vitamins, minerals, and trace elements.

Materials and Methods: Subjects met the diagnostic criteria for PKU, and informed consent was obtained from 29 children with PKU and 29 children from the control group.

Results: The total number of red blood cells (RBC), haemoglobin concentration (HG), haematocrit (HCT), and biochemical parameters (transaminases, total proteins, albumin, urea, uric acid, creatinine, triglycerides, total cholesterol, ionic calcium, and total calcium) do not show significant differences, except circulating iron level, which presented a statistical difference (p= 0.0005). When compared to the control group, a significant statistical difference was observed in the Vitamin A (p=0.0001) and Vitamin D (p=0.0026) levels but not in Vitamin E levels (p=0.1319). Also, we observed significant statistical differences for total iron (p-0.005), copper (p<0.0001), zinc (p=0.0903), manganese (p=0.0001), and chromium (p<0.0001) levels, but not for selenium level (p=0.7742).

The correlation between the amino acid levels, micronutrients, and biochemical parameters in patients with PKU showed a series of essential correlations that impact the state of health and the quality of life.

In conclusion, our studies indicate a complex and multifactorial relationship between the PKU pathophysiology and nutritional status that gives a satisfactory state of health. The guidelines that have begun to be developed as best practices for clinicians are based on the latest available evidence, but these cannot replace the physician's approach.

Keywords: PKU, nutritional status, liposoluble vitamins, iron deficiencies

ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS IN ACUTE AND CHRONIC INFLAMMATORY PROCESSES

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Background: Neutrophil extracellular traps (NETs) are structures composed of chromatin decorated with neutrophil-derived proteins that play a critical role in both the innate immune response and in various pathological conditions. Our work has expanded on the classical understanding of NET formation (NETosis) and its implications in both acute and chronic inflammation.

Methods: We have investigated the mechanisms and functional outcomes of NETs in both resolving and exacerbating inflammation. Our studies encompass in vitro and in vivo models of NET formation, focusing on the conditions under which NETs either promote resolution (via aggregated NETs, or "aggNETs") or contribute to disease pathology (via scattered NETs).

Results: i) Resolution of Inflammation via Aggregated NETs (aggNETs): Our research has demonstrated that aggNETs contain proteases, such as neutrophil elastase, that degrade pro-inflammatory cytokines, excluding IL-8. These structures are vital for resolving localized inflammation, particularly in tissue settings like gout or pancreatitis, where the aggregation of NETs effectively isolates and neutralizes inflammatory triggers. ii) Pathological Effects of Scattered NETs: In contrast, scattered NETs, particularly those produced in response to oxidative bursts, have been implicated in the pathogenesis of autoimmune diseases, cancer metastasis, thrombotic disorders and heart fibrosis. Our findings highlight the delicate balance required for efficient tissue protection by NETs from pathogen and tissue damage due to excessive NETs formation. iii) Induction of NETs by Nanoparticles: Unexpectedly, we discovered that inert nanoparticles, including both endogenous (cholesterol, monosodium urate) and exogenous (soot, nanodiamonds, aluminum oxide) particles, trigger NET formation. Additionally, when co-injected with antigens, these particles induce NETosis and potentiate immune responses, offering novel insights into nanoparticle-mediated vaccine adjuvants.

Conclusion: In inflammation, NETs act as mediators of resolution in tissue-specific contexts (via aggNETs) and drivers of pathology when dysregulated (*via* scattered NETs).

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ADVANCED NANOTHERAPEUTIC SOLUTIONS FOR CARDIOVASCULAR DISEASES

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Introduction: Cardiovascular diseases (CVD) are the leading cause of illness and death globally. A primary underlying cause of cardiovascular disease is atherosclerosis, a systemic condition caused by a complex accumulation of lipids, extracellular matrix, cells, and cellular debris accumulating in the artery walls. Recent advances in cardiovascular research have resulted in a notable change in focus toward addressing the resolution of chronic vascular inflammation observed within atherosclerotic lesions rather than just reducing cholesterol levels for atherosclerosis treatment.

Hypothesis: Nanomedicine approaches will lead to new therapeutic solutions for vascular inflammation in atherosclerosis.

Aim: To develop innovative nanoparticles that deliver various therapeutic agents to atherosclerotic plaques, reducing inflammation and slowing the progression of lesions.

Methods: We synthesized nanoparticles coated with peptides with a high affinity for cell adhesion molecules or biomimetic nanoparticles endowed with biological properties able to evade immune system recognition to enhance precision and minimize off-target effects.

Results: The findings from both in vitro and in vivo studies will be presented, focusing on several key aspects. These include the physicochemical characteristics of the developed nanoparticles, their potential for targeted delivery and accumulation within atherosclerotic plaques, the mechanisms of interaction with vascular and immune cells, their therapeutic effects, and safety assessments conducted in an animal model of atherosclerosis using ApoE-deficient mice.

Conclusion: With the help of nanomedicine, we can design nanosystems to target precisely the atherosclerotic plaque and deliver therapeutic agents to reduce plaque inflammation and promote inflammation resolution.

Acknowledgement: The support from Romania's National Recovery and Resilience Plan, PNRR-III-C9-2022-I8, CF 93/15.11.2022, Financing Contract no. 760063/23.05.2023 is acknowledged.

PRESENTATION SESSION 3

TUSC5, AN INTRACELLULAR GLUCOSE IMPORT MODULATOR EXPRESSED IN SENSORY GANGLION NEURONS

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We have previously proposed a combinatorial transcriptional code of Pou4f family factors in RGCs, and described the differentially expressed and regulated genes in these RGCs. Amongst them we have previously identified Tusc5 as a gene specifically expressed in Brn3a$^+$ RGCs and transcriptionally regulated by Brn3a/Pou4f1. We have generated a conditional knock-in reporter inversion-excision strategy targeted at the mouse Tusc5 locus, and use it to characterize Tusc5$^+$ RGC anatomic and physiologic properties. As previously reported, Tusc5$^+$ RGCs have dendritic arbors with small areas, and lamination restricted to the outer 66 % of the Inner Plexyform Layer.We report functional consequences of Tusc5 ablation in RGCs. We find that, besides RGCs, Tusc5 is expressed in Dorsal Root Ganglia (DRGs), Trigeminal Ganglion, Spiral and Vestibular Ganglion, and Olfactory neurons. In DRGs, Tusc5 expression subdefines specific functional classes of thermo and nociceptors. In addition, we present physiologic and biochemical evidence for Tusc5 function in energymetabolism.

UTILITY OF WHOLE EXOME SEQUENCING IN ROUTINE PRACTICE

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Introduction: Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) techniques significantly increased the knowledge of rare diseases and not only. By using such technology, we are closer to the principle of personalized medicine.

Material and Method: We used WES technology to our patients; we included in this study 100 patients that were analyzed by WES in the last year.

Results and discussion: The patients included in this study had different ages, from neonate to older adults, almost all with different, various, clinical backgrounds. The WES increased the positive results to 60% of our patients and a big part of the remaining needs to be annually reevaluated for Variant of Uncertain Significance (VUS). We will present and discuss rare syndromes such as Floating- Harbor Syndrome, Schuurs- Hoeijmakers Syndrome, KMT2B dystonia, cardiomyopathies and incidental findings that helped us to apply the principle of preventive medicine.

Conclusions: The WES and/ or WGS must be integrated in the diagnosis management of multiple patients, in the routine practice.

Keywords: WES, WGS, rare syndromes

UNRAVELING THE CELLULAR AND MOLECULAR MECHANISMS OF ELK3-DRIVEN METASTASIS IN TRIPLE-NEGATIVE BREAST CANCER

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Introduction: While metastasis is the leading cause of mortality in breast cancer, targeted therapies for this process remain elusive. Therefore, understanding the molecular drivers of metastasis is essential for developing therapies against metastatic breast cancer cells. In this context, this study investigates the role of the ELK3 transcription factor in the metastasis of triple-negative breast cancer (TNBC), focusing on the cellular and molecular processes it influences.

Materials and Methods: MDA231 TNBC cell line was engineered to either overexpress (OE) or knock down (KD) ELK3 using a lentivirus-assisted transduction procedure. Genetic modifications were confirmed by flow cytometry via GFP+ and validated through PCR and RT-qPCR. Whole transcriptome analysis of ELK3-KD cells was performed using the microarray technology to identify ELK3-modulated genes. RT-qPCR was employed to validate the microarray data. Ingenuity Pathway Analysis (IPA) software was used to predict metastasis-related cellular processes regulated by ELK3, and these predictions were validated in vitro. Migration was assessed using 3D microfluidic devices, stemness potential through mammosphere formation assays, and proliferation by the AlamarBlue assay.

Results: Over 95% of cells were successfully genetically modified in both ELK-OE and ELK-KD cell lines. Microarray analysis identified 765 genes modulated by ELK3. Two key cellular processes were predicted to be affected in ELK-KD cells: cell migration [upregulation of motor dysfunction; downregulation of cell movement, invasion, and actin organization] and stemness capacity [upregulation of cell cycle progression; downregulation of self-renewal capacity]. RT-qPCR validation highlighted HIF1a, FGFR1, NDGR1, BNIP3L, DUSP1, and PLAUR as crucial regulators of ELK3-mediated processes. Functionally, ELK3 modulation significantly impacted cell migration in 3D environments: overexpression increased migration speed (+31.2%) and persistence (+38%), while knockdown reduced both speed (-22.1%) and persistence (-23.4%). ELK3 also promoted stemness, as overexpression increased spheroid size (+17.8%) and number (+49.2%) in mammosphere formation assays. Additionally, ELK3 overexpression inhibited proliferation (-22% at 24h), whereas its knockdown promoted cell division (+29% at 24h).

Conclusions: ELK3 acts as a pro-metastatic transcription factor in TNBC, enhancing cell migration and stem-like traits while inhibiting proliferation. These findings suggest that if further validated in in vivo and clinical studies, ELK3 could be a promising target for migrastatic therapies in TNBC.

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ANTI-ROR1 CAR T CELLS IN MANTLE CELL LYMPHOMA

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Introduction: As a receptor for Wnt5a and other Wnt proteins, ROR1 regulates cell migration, differentiation and growth during embryonic development. It is demonstrated that ROR1 expression is low in adult tissues like pancreatic islets, adipose tissue or lungs, and in some cases even undetectable. The presence of ROR1 was reported in multiple tumor tissues, with different expression grades. Mantle Cell Lymphoma (MCL) is a highly ROR1 positive tumor and is characterized as a very aggressive and rare form of non-Hodgkin Lymphoma (NHL) originating in the mantle zone of lymph nodes. The main objective of this study is to develop a novel CAR T cell based targeted therapy, focused on ROR1 and to optimize it on MCL in vitro models.

Materials and Methods: The CAR T cell was generated by inserting the plasmid via lentiviral vector created by Creative Biolabs. The cell culturing was performed using media, supplemented with glutamine, FBS or HS, IL-2 and/or Dynabeads. Z138 cell line was used as MCL model, and the ROR1 expression was confirmed by Flow cytometry. The co-culture between CAR T and Z138 included different E:T ratios and different time point for evaluation. For CAR T cell efficacy, the cytokine release was measured by ELISA and the LDH activity by PicoProbe assay kit. The ROR1 CAR plasmid was inserted into Jurkat cell line (T-cells) by viral transfection. The CAR T cell eGFP sorting was performed on FACS Aria Flow cytometer and the bright eGFP population was isolated and expanded.

Results: As immortal CAR T cells, the adjusted protocol started with CAR T activation of with Dynabeads targeting CD3/CD28 and then cocultured with target cells. After 24h, 72h and one week, the cells were evaluated by Flow cytometry and the cell culture supernatant was stored for Cytokine release analysis and LDH activity evaluation. The anti-ROR1 CAR T cells displayed a time-dependent inhibitory effect on Z138 cells, with IL-6 and TNFa release after coculture. Also, at 24h coculture, the LDH activity was increased in all groups that contained CAR T cells. The experiments were performed in comparison with Mock cells (Jurkat cells).

Conclusions: The novel anti-ROR1 CAR T cells have limited inhibitory effect against MCL, due to low activation and cytotoxicity and needs further investigation to improve the efficacy of CAR T cells.

ROLE OF NEUTROPHIL SUBTYPES IN PHENOTYPIC MODULATION OF FIBROBLAST AND MACROPHAGES DURING CARDIAC INJURY AND REPAIR POST-MYOCARDIAL INFARCTION

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Introduction: Neutrophils, traditionally regarded as a homogenous population, are pivotal first responders in host defense, driving the innate immune response. Recently, they have been phenotypically classified into pro-inflammatory N1 and anti-inflammatory N2 subtypes, though their distinct functional roles remain poorly understood. This study explored the functional and phenotypic differences between N1 and N2 neutrophils and their impact on fibroblast and macrophage phenotypes and functions during cardiac injury and repair following myocardial infarction.

Materials and Methods: N1 and N2 neutrophils were obtained by in vitro polarisation using 100 ng/ml lipopolysaccharide and 20 ng/ml IFNγ (N1) or 20 ng/ml interleukine-4 (N2) and analyzed by RNA-Seq. Fibroblasts were co-cultured with N1/N2 neutrophils using a two-chamber Transwell system and macrophages were incubated with the secretome of N1/N2 neutrophils. The fibroblast and macrophage were assessed by evaluating the expression of different inflammatory, remodeling, pro-fibrotic, and efferocytic molecules using qPCR, Western Blot, and ELISA assay. Cell functionality was investigated by xCELLigence Real-time analysis, wound healing, and efferocytosis assay.

Results: We identified distinct transcriptomic and functional differences between N1 and N2 neutrophils. N1 neutrophils, compared to N2, exhibited: i) elevated ROS production and oxidative burst, ii) increased MPO and MMP-9 activity, and iii) heightened chemotactic response. Additionally, N1 neutrophils showed upregulated expression of NADPH oxidase subunits and activation of ERK1/2 and the NF-kB p65 subunit. Cross-talk between cardiac fibroblasts and N1 induced a pro-inflammatory and matrix-degrading phenotype to fibroblast, delineated by overexpression of inflammatory cytokines IL-6, MCP-1, IL-1β, MIP-1α, RANTES, and metalloproteases MMP-1 and MMP-9. Furthermore, macrophages exposed to the N2 neutrophil secretome demonstrated: (i) elevated expression of anti-inflammatory markers TGF-β, CD206, and IL-10; (ii) upregulation of efferocytosis receptors (MerTK, CX3CR1, CD36, integrins αv/β5) and bridge molecules Gas6 and Mfge8; and (iii) improved efferocytosis efficiency.

Conclusion: These findings demonstrate that N1 neutrophils act as pro-inflammatory mediators of the innate immune response. Distinct neutrophil subtypes likely drive changes in fibroblast and macrophage phenotypes following myocardial infarction, thereby regulating the post-MI reparative process.

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Keywords: N1/N2 neutrophils, macrophages, fibroblasts, inflammatory molecules, efferocytosis

POSTER SESSION

CHITOSAN TYPES INFLUENCE ON YKL-40 MELANOCYTES EXPRESSION

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Introduction: The cellular activity of chitosan has been studied in various types of cancer, including melanoma, and indicated that these molecules can open new perspectives on antiproliferative action and anticancer therapy. Our study analyses how different chitosan conformations, such as α-chitosan or β-oligochitosan, both in different concentrations and in mixtures, influence the cellular processes of SK-MEL-28 melanocytes.

Materials and Methods: α-chitosan and β-oligochitosan with different values of deacetylation degree (DDA) and molar mass (MM) were investigated. The expression levels of YKL-40, chitinase-like proteins, were assessed by the ELISA assay kit.

Results: Our results showed a decrease in tumor cell viability and YKL-40 expression in the association conditions of α-chitosan–β-oligochitosan mixtures after SK-MEL-28 melanocytes stimulation. While DDA is a critical factor in the magnitude of the effects, MM influences both the penetration of chitosan and the speed of the induced reactions. The level of YKL-40 decreases in the tested solutions from 7% to 28% when the mixture of α-chitosan and β-oligochitosan (MM<1.5 kDa) is added compared to the control sample.

Conclusion: The different chitosan types and their influence on the expression of the chitinase-like protein YKL-40 on SK-MEL-28 melanocytes may open new alternatives for tumor inhibition, being a promising prospective direction.

AN OPTIMIZED MACHINE LEARNING-BASED APPROACH FOR AUTOMATED ANALYSIS OF INSULIN GRANULES ON ELECTRON MICROGRAPHS

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Background: Dysfunctional insulin secretion by pancreatic β-cells, a hallmark of diabetes, is associated with cellular stress. Understanding of the early defects in the insulin secretory pathway is critical and insulin granule quantification could provide a valuable insight.

Aim: to develop a machine learning-assisted method for the automated quantification of insulin granules, addressing the complexity and heterogeneity of electron microscopy (EM) images.

Methods: Small pancreatic fragments were collected from mice in a model of induced β-cell stress (n=3 per time point: days 0, 1, 3, and 5 after stress induction), processed for standard EM, and imaged. For the automated analysis of insulin granules, we developed a workflow integrating two open-source frameworks, FIJI/ImageJ and ilastik (an interactive, machine learning-based tool), with Python. The processing sequence includes: (i) splitting images into quadrants to improve computational efficiency, (ii) preprocessing in FIJI to highlight granule features, (iii) converting the preprocessed images into ilastik-compatible formats using Python scripts, (iv) training and applying a feature-based classifier in ilastik to segment images based on texture, edges, and intensity patterns, (v) exporting the segmented results and reformatting them for subsequent analysis in FIJI, and (vi) applying thresholding and binarization to enable object detection with FIJI's particle analysis function, followed by measurements of granule number, size, area, and intensity.

Results: In EM images, insulin granules appear as electron-dense cores (crystallized insulin) surrounded by a halo and an enclosing membrane. Preliminary results show that our streamlined workflow effectively segments dense-core granules and their halos in large image datasets, facilitating quantitative analysis of granule numbers, morphology, and spatial distribution, as evidenced by binary mask overlays on the original EM images. The data collected thus far reveal notable differences between the experimental groups.

Conclusion: This machine learning-based approach provides a valuable tool for analyzing changes in the insulin secretory pathway under stress and could lead to novel insights into early compensatory mechanisms in diabetes progression.

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PROTEOMIC INSIGHTS INTO DIABETIC PERIPHERAL ARTERIAL DISEASE LESION. PRELIMINARY RESULTS

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Introduction: Peripheral arterial disease (PAD) often leads to severe foot lesions, impacting patient quality of life and requiring high costs intervention.

Aim: To identify early therapeutic targets and resume disease progression in diabetic PAD patients based on clinical data and proteomic analysis.

Materials and Methods: Data on symptoms, pulse presence in various arteries, and foot lesion stages were collected from 17 PAD patients, classified by the Wagner-Armstrong system. A proteomic analysis of dorsalis pedis vascular tissue was performed.

Results: Among the 2314 identified proteins, 21 Damage-Associated Molecular Pattern (DAMP) molecules showed significant changes between different lesion stages (Wagner W5 vs Wagner W3). Principal Component Analysis revealed distinct proteomic profiles for stages W3 versus W5. Severe arterial lesions displayed up-regulation of Biglycan (BGN); heat shock proteins (HSPD1, HSP90AB1, HSPA7), Neutrophil defensins, Decorin and isoforms, Tenascin-X and isoforms, Serum amyloid A-1 protein, Histone H3.3C. Interestingly, we detected down-regulation of Fibrinogen, Isoform 3 of (F-actin)-monooxygenase MICAL3, Histone H1.5, HSP90B1 in W5 reported to W3. Key KEGG pathways included complement and coagulation cascades, extracellular matrix organization, and peptide cross-linking were significantly over-represented. In the blood collected from patients with PAD, clinical parameters were altered. HSPA7 expression negatively correlated with some blood cells count (leukocytes and neutrophils) and positively with lymphocytes. BGN expression negatively correlated with red blood cell count.

Conclusions: Comparing foot lesions using clinical and proteomic data highlights the PAD progression that require urgent new designed therapeutics. Identified DAMP molecules suggest valuable intervention targets, while tailored therapies based on lesion severity and molecular profiles can improve PAD management.

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NON-CODING RNAS AS NOVEL BIOMARKERS IN ATHEROSCLEROSIS

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Introduction: Atherosclerosis is the main feature of cardiovascular disease (CVD), despite tireless technological and medical development. There is an intensive effort to identify biomarkers to predict atherosclerotic lesions progression.

Hypothesis.: We hypothesized that there are some epigenetic markers, such as microRNAs (miRNAs), which could be indicators for the presence of atherosclerotic multiple vascular pathology (MVP) in patients with carotid artery stenosis (CAS) or peripheral artery disease (PAD).

Aim: We planned to determine the potential of six selected miRNAs, known to be involved in CAS and PAD disorders (miR-223-3p, miR-142-3p, miR-155-5p, miR-122-5p, miR-486-5p, and miR-92a-3p) to be diagnostic biomarkers for the atherosclerotic MVP.

Materials and Methods: 32 CAS patients and 55 PAD patients were enrolled for this study. Plasma levels for the six miRNAs were assessed by RT-qPCR in all CAS and PAD patients, and analyzed for statistical association with atherosclerotic MVP diagnosis.

Results: Significant increases for plasma miR-223-3p, miR-142-3p, miR-155-5p, miR-122-5p, and miR-92a-5p levels were determined in CAS patients with MVP compared to those without MVP. In PAD patients, MVP diagnosis was associated with significant increases of plasma miR-223-3p, miR-486-5p and miR-92a-5p levels, while plasma miR-142-3p, miR-155-5p and miR-122-5p levels were not significantly modified. In CAS patients, plasma levels of miR-92a-3p, miR-486-5p, miR-223-3p, miR-122-5p and miR-155-5p were identified as strong independent predictors for MVP presence in univariate ROC analysis adjusted for age and gender. In PAD patients, only miR-486-5p was identified as a strong independent predictor for MVP in univariate ROC analysis adjusted for age and gender. We established that a combination of two plasma miRNAs, miR-223-3p and miR-486-5p, is the best minimal significant multivariate ROC model that can significantly predict atherosclerotic MVP occurrence in both CAS and PAD patients.

Conclusion: We propose miR-223-3p and miR-486-5p as effective new epigenetic biomarkers for atherosclerotic MVP diagnosis in both CAS and PAD patients. They could also be robust predictors for the evolution of atherosclerotic vascular disease or metabolic disorders.

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VARIATION OF SOME INFLAMMATORY BIOMARKERS IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS

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Introduction: Osteoarthritis, the most common type of arthritis, is a degenerative disease rising when joint cartilage and underlying bone break down followed by joint stiffness. Bee venom (BV) can be assumed as a nutraceutical rich in active compounds, such as peptides and enzymes, having several pharmaceutical features, including anti-inflammatory activity. In view of its anti-inflammatory properties, the present study was designed to assess the antiarthritic effect of BV samples in a Freund's complete adjuvant (CFA) induced knee osteoarthritis (KOA) model in rats.

Materials and Methods: BV samples were collected in beehives from Călărași area, Dolj county. BV was administrated i.p. twenty-one consecutive days after inducing KOA in one group of rats with a 1 mg/mL CFA injection in the knee joint and methotrexate (MTX) once a week in another group. The negative control were rats receiving sterile saline solution and the positive one, a group of untreated with BV or MTX rats receiving CFA. At the end of the treatment, blood levels of MPO, TNF-α, IL-1β and anti-mutant citrullinated vimentin (MCV) antibodies were measured. Joint tissue fragments obtained after decalcification and formalin fixation were processed for paraffin embedding and then for hematoxylin-eosin and toluidine blue staining techniques.

Results: BV and MTX had the tendency to decrease the level of MPO activity but without any statistically significant difference. Both BV and MTX decrease the levels of inflammatory biomarkers TNF-α and IL-1β but they didn't restore them to the normal levels. BV and MTX decreased also the levels of antibodies against MCV, a molecule that indicates the gravity of changes in the case of arthritis, which revealed a significant increased level in CFA-treated rats in comparison with negative controls. Sections of joints from CFA-treated rats showed the loss of substance and proteoglycans content in extended areas (Mankin score 5-6); for BV and MTX treated rats, the structural changes were diminished (Mankin score 3).

Conclusion: Overall, our results showed that BV adjuvant therapy may be a substitute for MTX treatment in order to reduce inflammation and improve health status in KOA but is important to adjust its therapeutic dose.

TOTAL PHENOLIC CONTENT, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SOME *SALVIA* SPECIES HARVESTED FROM THE OLTENIA REGION

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Introduction: Plants were used as traditional medicine since ancient times and, recently, there has been an enhancing interest towards the potential biological activities of plant derived compounds. Therefore, the aim of this work was to assess the antioxidant capacity along with the antimicrobial activity of the ethanolic extracts obtained from three different ***Salvia*** species, whilst screening their content of total phenols (TPC) and flavonoids (TFC).

Materials and Methods: Three *Salvia* species, namely ***S. aethiopis, S. nemorosa*** and ***S. verticillata*** were harvested from the Oltenia region during flowering period. Their dried material was powdered and ethanolic extracts were obtained. The quantification of TPC and TFC was performed using Folin-Ciocâlteu assay and aluminium trichloride method. Free radical scavenging activity was evaluated using DPPH and ABTS **Methods:** Using disk diffusion technique and minimum inhibitory concentration, the antimicrobial activity was evaluated against standard bacterial and fungal strains - ***S. aureus***, ***E. faecalis*** and ***C. albicans,*** respectively.

Results: *S. verticillata* ethanolic extract possessed the highest polyphenolic content (73.62±1.09 mgGAE/g d.w.) comparing to ***S. nemorosa*** (64.86±1.82mg GAE/g d.w.) and ***S. aethiopis*** (47.10±2.76 mgGAE/g d.w.). TFC ranged from 4.51±0.18 to 6.72±0.43 mg QE/g d.w. the value for ***S. nemorosa***. The extracts exhibited antioxidant activity with ***S. aethiopis*** the strongest in DPPH method - IC_{50} of 12.84±1.10 µg/mL. The antioxidant activity of ***S. nemorosa*** was the strongest in the $ABTS^{\bullet+}$test (an IC_{50} of 18.52±1.70 µg/mL). All ethanolic extracts inhibited the growth of the clinically important tested strains. Inhibition diameters of ***E. faecalis*** growth ranged from 11 to 12.33±0.58 mm, with ***S. verticillata*** being the most active. ***S. aethiopis*** showed the highest antibacterial activity against ***S. aureus*** (16.33±0.58 mm) and the most efficient antifungal activity was shown by ***S. nemorosa*** (15.66±0.58 mm).

Conclusions: The results suggested that the antioxidant and antimicrobial activities are correlated with the TPC and TFC and these Salvia species could qualify for use as an adjuvant solution in various infections caused by Gram positive bacteria and fungi.

TOXICOLOGICAL CONSIDERATIONS OF PHYTOCHEMICALS USED IN CANCER TREATMENTS

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Introduction: With both incidence and mortality rates on the rise, cancer is a leading cause of death in developing countries. Although traditional methods of treating cancer have improved over time, we cannot ignore the myriad of side effects, the lack of more potent drugs and high cost of therapy, important factors that dramatically decrease the quality of life of patients and insignificantly growth of survival rates. In this context, there is an increasing use of herbal bioactive compounds, with the main purpose of alleviating symptoms and presenting a valid alternative in cancer treatment. Some phytochemicals are potential carcinogens or tumor promoters, therefore it is necessary to analyze both benefits and risks to properly assess the safety of their use in cancer treatment.

Materials and Methods: This paper reviews the novel therapeutic potential and toxicological considerations of phytochemicals as alternative therapies in cancer treatment.

Results: Toxicity of plant compounds is unfortunately understudied, and the myth that consuming medicinal plants or plant-derived remedies is beneficial to health is partially erroneous. Up-to-date studies highlight the risks that arise from the interaction between some phytochemicals and chemotherapeutic agents, causing inadmissible side effects. Also, several well-known anticancer phytochemicals present limited bioavailability and stability, which increaseas their curative potential.

Conclusions: Most phytochemicals reduce the risk of cancer, due to antioxidant and anti-inflammatory effects, but the concerns regarding the lack of validation in randomized human clinical trials and toxicological issues should not be neglected. Plant derived compounds need to be investigated in order to represent an effective alternative cancer treatment option, by elucidating the balance between beneficial and toxic effects.

PENTOSE PHOSPHATE PATHWAY DISRUPTION IN PARKINSON'S DISEASE: A METABOLIC LINK TO NEURODEGENERATION

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Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra. While the exact mechanisms underlying PD remain unclear, metabolic dysregulation and oxidative stress are key contributors. The pentose phosphate pathway (PPP), a metabolic route primarily responsible for producing ribose-5-phosphate and reducing equivalents (NADPH), plays a pivotal role in glucose metabolism and cellular redox balance. However, the relationship between the PPP and PD pathology remains largely unexplored. In particular, the roles of ribose and xylose, key products of the PPP, have not been thoroughly investigated in the context of neurodegeneration.

This study aims to bridge this gap by examining the metabolic alterations in PD, with a focus on PPP-related metabolites.

Materials and Methods: Parkinson's disease was induced in CD21 mice through a dopaminergic lesion using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at a dosage of 25 mg/kg body weight, administered intraperitoneally (i.p.). The experimental groups consisted of MPTP-treated and control mice. Following treatment, the brains were dissected, and the cortical and striatal regions were isolated for analysis. The assays included G6PDH activity assay, oxidative and reductive stress markers, and metabolomic profiling focusing on the PPP. Particular attention was given to ribose and xylose concentrations, as they represent key PPP metabolites.

Results: Analysis of the cortical and striatal regions revealed significant alterations in oxidative stress markers and enzyme activity in the MPTP-treated group compared to controls. Metabolomic profiling showed a statistically significant increase in ribose and xylose levels in the PD model ($p < 0.05$). These findings suggest a potential dysregulation of the pentose phosphate pathway in the presence of dopaminergic degeneration, involving PPP intermediates in the metabolic dysfunction observed in PD.

Conclusion: The study highlights the involvement of the pentose phosphate pathway in Parkinson's disease pathology. Increased levels of ribose and xylose in the PD model suggest that metabolic dysregulation, particularly through the PPP, may contribute to oxidative stress and neuronal degeneration in PD. These findings provide a new link between glucose metabolism and neurodegeneration and open new perspectives for targeted therapeutic strategies.

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ECOLOGICAL APPLICATIONS IN PLANT WASTE RECYCLING

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Defining waste is difficult due to its variety and complex composition. The EU Directive define waste as "any substance or object which the holder discards" (Directive, 2008), considering the waste as something of no value for humans.

In the context of growing concerns about sustainability and resource efficiency, the valorization of plant residues resulting from the production of various plant extracts has become a particularly relevant research and development topic. Extraction processes to obtain bioactive substances, essential oils, or other compounds of pharmaceutical interest, can generate significant amounts of unused plant materials, also called plant waste.

Examples of methods for recovering plant waste:

1. Valorization of wet olive pulp (WOP) in the tannery industry. WOP contains a lot of polyphenolic substances, which give it a use as a skin tanning agent. 39.6% of the polyphenols in WOP are tannins, capable of tanning the skin, and 14.3% correspond to non-tannins, molecules that do not have the ability to tan the skin but contribute to the skin's tanning mechanism and increase the quality of tanned leather.

2. Valorization of citrus waste in the pharmaceutical industry. Citrus fruits, such as oranges, grapefruits, lemons, and tangerines are among the most popular fruits grown across the globe. The genus ***Citrus*** (***Rutaceae*** family) it contains bioactive compounds and nutrients that give it health benefits. These natural compounds have antibacterial, anti-inflammatory, antineoplastic and antidiabetic activities. Citrus processing industries generate waste that has an important economic value due to the content of bioactive compounds that can be used in pharmaceutical and nutraceutical formulations.

CHRONIC INFLAMMATION RELATED TO RHEUMATOID ARTHRITIS INCREASES THE RISK OF CARDIOVASCULAR DISEASE

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Rheumatoid arthritis (RA) is the most prevalent form of autoimmune arthritis, characterized by symmetric involvement of multiple joints, frequently accompanied by systemic manifestations. RA is now considered an independent risk factor for cardiovascular disease. Chronic inflammation associated with RA is responsible for the increased risk for atherosclerotic cardiovascular disease observed în RA patients. Involvement of neutrophils and proinflammatory cytokines play a key role in the pathogenesis of RA. The high levels of oxidative stress related to the chronic inflamation in RA might lead to vascular injury. The proinflammatory state also leads to the disruption of vascular homeostasis and endothelial dysfunction. In turn, endothelial dysruption is the earliest stage of atherosclerotic disease, and has been observed even in patients with incipient RA, regardless of the presence of other classical cardiovascular risk factors.

We present the case of a 76 years-old male patient with stage II seropositive RA of functional class I/II and systemic manifestations. Autoimmune arthritis engages symmetrical involvement of hand joints, as well as the elbows, scapulo-humeral joints, knees and spine. The patient is a former smoker, of normosthenic constitution (BMI = 24 kg/m^2). An estimated glomerular filtration rate of 43 ml/min/ 1.73m^2 is indicative of chronic kidney disease stage IIIb. Lung involvement by RA in this patient is in the form of diffuse intersitial pulmonary fibrosis, manifesting as mild respiratory impairment. The active inflammation process is reflected by the high levels of serum C-reactive protein (40 mg/l). The lipid profile is maintained within desirable ranges by the use of rosuvastatin treatment. Cardiovascular disease in this patient developed as ischemic heart disease and essential arterial hypertension. Our patient has a history of myocardial infarction followed by a coronary stent insertion in February 2021 and a mesenteric stent insertion in February 2022. The QRISK3 estimated risk of a major cardiovascular event within the next 10 years in this patient is 37%, translating into a relative risk of 1.5 compared with that of a healthy person of the same sex and age. However, the QRISK3 score does not take into account the history of myocardial infarction and the advanced stage of atherosclerotic disease necessitating the mounting of two stents in different arterial systems. It is thus much likely that the cardiovascular risk is considerably higher than predicted by the QRISK3 score.

THE RELATIONSHIP BETWEEN THE NUMBER OF CUMULUS CELL LAYERS AND *IN VITRO* MATURATION OF OOCYTES IN PIGS

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Introduction: The process of in vitro maturation of oocytes is a complex one that involves a sequence of processes occurring both at the level of the nucleus and in the cytoplasm of the oocyte. The maturation of the oocytes must be carried out efficiently, being an essential stage for the acquisition of developmental competence after the oocyte has been fertilized. An essential role in the oocyte maturation process is played by the surrounding somatic cells, cumulus cells. The cumulus cells are in direct contact with the oocyte, through functional gap junctions, through the surrounding zona pellucida. In case of premature loss of cumulus-oocyte communication, the in vitro maturation of bovine oocytes and post-fertilization blastocyst development is affected. The purpose of the study was to structurally and functionally analyze the oocyte-cumulus complexes, respectively to monitor the in vitro maturation process of these oocyte-cumulus complexes.

Materials and Methods: In this sense, 20 pig ovaries were used, obtained from slaughterhouses after slaughtering the scrophytes. From the ovaries taken in the study, 282 ovarian follicles were highlighted and isolated from which 246 oocyte-cumulus complexes were isolated and extracted, which presented different organization. The isolated oocyte-cumulus complexes were subjected to maturation in vitro, in a specific culture medium - TCM-199 culture medium.

Results and Conclusions: The in vitro maturation process of the oocytes extracted from these complexes was realized differently, the recorded maturation rates being influenced by the number of layers of cumulative cells. In the conducted study, the highest maturation rate of 92.12% was highlighted at the level of oocyte-cumulus complexes where several layers of cumulus cells were identified. However, the process of fertilization and embryonic development carried out in vitro was relatively low, recording rates of 10.61%, values recorded for oocytes matured in vitro and extracted from those oocyte-cumulus complexes with several layers of cumulus cells.

TESTING THE ANTIBACTERIAL POTENTIAL OF *THYMUS PULEGIOIDES* EXTRACTS

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Introduction: Worldwide, there is an obvious increase in antibiotic resistance, for this reason many questions have appeared regarding the continuation of the administration of existing antibiotics on the market, the synthesis of antibiotics with a broad spectrum of action and with a higher concentration of active compounds. Recently, special attention has been given to plant extracts that, thanks to their biologically active compounds, exhibit antiviral, antibacterial, antifungal, antitumor, antiseptic, antioxidant, antiproliferative properties. In this sense, extracts from Thymus pulegioides have started to be used for medical purposes, precisely because of their antimicrobial properties due mainly to the biologically active compounds in the extracts. T. pulegioides extracts contain: volatile oils (bomeol, cineol, thymol, cimol, carvacrol, α-terpineol, β-caryophyllene, myrcene, etc.), tannin, flavonic derivatives, ursolic acid, caffeic acid, serpilin, mineral salts, etc. The phytotherapeutic importance in human and veterinary medicine is due to its cholagogue, stomachic, choleretic, anthelmintic and antiseptic, cicatrizing, antidiarheal, diuretic and antiviral, antiseptic, antispasmodic and antimicrobial properties. The aim of the present study was to evaluate the possible potential of the ethanolic extracts made from the aerial parts (leaves and stem) of T. pulegioides harvested from the Bihor region (Romania).

Materials and Methods: The antimicrobial potential is directly related to the biologically active compounds obtained in the extract, which are identified in different concentrations depending on the geographical area of origin of the plants. Microbiological tests were performed using standardized bacterial strains: Gram+ (Staphylococcus aureus and Streptococcus pyogenes), respectively Gram- (Escherichia coli).

Results and Conclusions: The antibacterial potential of the ethanolic extracts of T. pulegioides was realized using cell viability assays. The ethanolic extracts of T. pulegioides showed different antibacterial potential depending on the tested concentration, the vegetative organ from which the extract was made and last but not least the bacterial strain studied. The decrease in the concentration of T. pulegioides extract causes a decrease in the antibacterial potential from bacteriolytic to bacteriostatic, until the absence of the antibacterial effect in most bacterial strains. The values of the cell viability tests highlight a more obvious antibacterial potential in Gram+ bacteria compared to Gram-.

ASSESMENT OF THE 16S-23S ITS AND CPCBA-IGS AS PHYLOGENETIC MARKERS FOR *MICROCYSTIS* STRAINS (CYANOBACTERIA)

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Introduction: The aim of our study was to explore suitability of the 16S-23S ITS and the cpcBA-IGS sequences as phylogenetic markers for the cyanobacteria genus Microcystis. For this purpose the phylogenetic relationships between 22 Microcystis strains from the AICB culture collection at the Institute of Biological Research, Cluj-Napoca, Romania were explored.

Materials and Methods: The AICB strains were grown on BG-11 liquid medium; genomic DNA was then extracted and used for the amplification of the 16S-23S ITS and cpcBA-IGS fragments. Resulting PCR amplicons were purified and sequenced bidirectionally. The obtained sequences were used in phylogenetic analyses together other with sequences from international databases (GenBank).

Results and Discussions: Several phylogenetic methods were used to analyze the sequence sets. The topology of the trees obtained was very similar, therefore only the maximum likelihood tree was used. The 22 AICB strains showed two main clusters for cpcBA-IGS and 16S-23S ITS markers. Also Microcystis flos-aquae and Microcystis aeruginosa strains formed one cluster in the 16S-23S ITS tree.

Conclusions: The AICB strains were grouped into two main clusters for both molecular markers. Microcystis aeruginosa and Microcystis flos-aquae formed one cluster in the 16S-23S ITS tree. This result supports their previous classification into a single genetic species (the Microcystis aeruginosa complex). Since the phylogenies based on the cpcBA-IGS and 16S-23S ITS are in good agreement, we find the two markers as good molecular tools to be used in the taxonomy of the Microcystis genus.

Keywords: cyanobacteria; Microcystis; 16S-23S ITS; cpcBA-IGS, phylogeny.

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RELATIONS BETWEEN PSYCHOLEPTIC MEDICATION AND PROLACTINE VALUES

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Introduction: Hyperprolactinemic syndrome presents with various etiopathogenic forms, the most frequent being polycystic ovary syndrome (PCOS), prolactin-secreting pituitary tumors (prolactinomas), administration of psycholeptic drugs, primary hypothyroidism, idiopathic forms, and other pituitary tumors, such as adenomas with mixed GH and PRL secretion. The clinical symptomatology includes features common to all forms, as well as specific manifestations with variable severity.

Material and Method: The study included patients with hyperprolactinemia induced by psycholeptic medications such as risperidone (4-7.6 mg/day), haloperidol (5-12.9 mg/day), olanzapine (10-17.5 mg/day), and ziprasidone (160 mg/day). Plasma prolactin levels and clinical symptoms were monitored over a period of 7-10 days. The results showed that prolactin levels were higher with risperidone treatment (above 90 ng/ml) compared to other medications such as haloperidol or olanzapine. Hyperprolactinemia improved after 7-10 days of treatment, with subsequent stabilization of prolactin levels.

Results: Our findings demonstrate that risperidone treatment generated the highest increases in plasma prolactin levels, and dose reduction, medication substitution, or the use of a dopamine agonist were effective solutions for managing elevated levels. The specialist team adjusted treatment based on the evolution of symptoms and the severity of the syndrome. Treatment of iatrogenic hyperprolactinemia was effective, and clinical improvement correlated with the reduction in prolactin levels.

Conclusions: Thus, careful monitoring of prolactin levels and personalized treatment are essential in managing hyperprolactinemic syndrome, especially in cases induced by psycholeptics, to minimize its impact on patients and ensure optimal therapeutic efficacy.

Keywords: Hperprolactinemic syndrome, prolactinomas, psycholeptic drugs, plasma prolactin levels, personalized treatment.

THE CORRELATION BETWEEN ANGIOGENESIS AND THE DEGREE OF TUMOR DIFFERENTIATION IN RENAL CARCINOMAS

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Introduction: Angiogenesis represents one of the important factors of tumor proliferation. Theoretically, an intense neovascularization allows a rapid tumor growth, while the decrease of angiogenesis process will reduce or even stop the neoplazic growth. In our study we tried to establish a correlation between angiogenesis and the degree of tumor differentiation in renal carcinomas.

Material and Method: A number of 43 patients were monitored, which had operated renal tumors, in the Department of Urology of the County Hospital Timisoara. The surgical treatment performed was total nefrectomy. The surgical excision pieces were processed by the Morphopathology Laboratory of County Hospital Timisoara and the Histopathology Laboratory of the Department of Morphopathology from University of Medicine and Pharmacy "Victor Babes" Timisoara. In order to reveal the angiogenesis, the tissue samples were processed by usual methods (paraffin inclusion, several sections were made, hematoxylin-eosin staining), and by special methods of immunohistochemistry for CD31, using CD31 DACO monoclonal antibody; for revealing we used the DACO peroxidase system LSAB2, the chromogen being DAB.

Results: From the 43 tumors, 9 of them well differentiated in G_1, 27 of them in G_2 and 7 tumors were weakly differentiated in G_3. The relative vascular density determined by microscopy, showed the highest values in case of G_1 renal carcinomas, of 27,35. The lowest vascular density, of 4,95, was archived for the less differentiated carcinomas. For G_2 tumors, the relative vascular density was 11,98.

Conclusions: The relative vascular density assessed by immunohistochemical reaction to CD31, indicates an inverse proportionality between the tumor differentiation degree and vascular density.

ASSESSING THE THERAPEUTIC POTENTIAL OF ZINC COMPOUNDS

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Introduction: Periodontal diseases are major public health problems worldwide, affecting more than 50% of the global population, resulting in high oral health care costs. The molecular events involved in the development of periodontal disease involve the formation of microbial biofilm by pathogens, leading to an immune response from the host. The aim of the study was to evaluate the biocompatibility and antiproliferative effect of the zinc compound on human cells.

Material and Method: [Zn3(HL)2(H2O)6](SO4) is a synthetic compound, testing was done with five different concentrations of the compound. The human biological material was represented by the cell lines MSC (mesenchymal STEM cells) and HdFa (human dermal fibroblast). The cells were incubated with the tested compound concentrations for 24h and 72h hours. After incubation, cell proliferation was determined by the MTT colorimetric technique for cells cultured in monolayer (2D). Cell morphology was analyzed using a Zeiss Axio Observer A1 Inverted Phase Contrast Microscope (Zeiss).

Results and Conclusions: MTT assay revealed that no significant reduction in cell proliferation rate was detected upon exposure of human MSC and HdFa cells at any of the concentrations tested. The values observed were between 99.98% and 100.2% compared to control cells, untreated cells. Also, based on the microscopy analyses, no changes in the cellular phenotype were identified.

PROTEINS INVOLVED IN CARDIAC ATP PRODUCTION AND CONTRACTILITY ARE MODULATED BY SHORT-TERM S100A9 BLOCKADE AFTER MYOCARDIAL INFARCTION

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Introduction: The infarcted heart is energetically compromised exhibiting a deficient production of ATP and the ensuing impaired contractile function. Others and we have previously revealed that short-term blockade of the protein S100A9 increases cardiac performance in mice after myocardial infarction (MI). The implications upon ATP production during this process are not known. In this study we aimed to evaluate whether S100A9 blockade has an effect on ATP synthesis and cardiac contractility after MI.

Methods: Thirteen C57BL/6 mice were divided into three experimental groups: (i) mice with MI, (ii) mice with MI treated with ABR-238901, a pharmacological blocker of S100A9, and (iii) sham (control) mice. MI was induced by permanent left coronary ligation. Mass spectrometry, pathway enrichment analysis, Western blot, and RT-PCR were performed on left ventricle tissues harvested 7 days post-MI from experimental groups.

Results: We detected 958 proteins in left ventricle samples of Sham, MI and MI+ABR mice with a Sequest Score ≥ 10 and unique peptide matches ≥ 2. Compared to the MI group, in the MI+ABR mice the abundance of 600 proteins was significantly altered (abundance ratio ≥ 1.5 or ≤ 0.667; adjusted P-value ≤ 0.05). The pathway enrichment analysis revealed the association of several of these proteins with oxidative phosphorylation, mitochondrial fatty acid beta-oxidation, citrate cycle, and cardiac muscle contraction. The S100A9 blocker also increased in the ischemic ventricle the abundance of several proteins (NDUFAB1, UQCRC1, HADHA, ACAA2, ALDOA, PKM1, DLD, DLAT, PDHX, ACO2, IDH3A, FH1, CKM, CKMT2, ATP2A2) which are important for ATP production and distribution and contractility.

Conclusion: This study provides direct evidence that blocking S100A9 in the first 48 h post-MI significantly improves ATP production. The data expand the knowledge of the critical players involved in the recovery of energy metabolism post-MI.

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NETOSIS INHIBITOR TREATMENT HAVE ACTIVE EFFECT ON THE DIABETIC LIVER RECOVERY

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Introduction: Non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) are closely connected through shared mechanisms such as insulin resistance and chronic inflammation. This study aimed to investigate the role of neutrophils, key phagocytes of the innate immune system, as a potential link in the development and interplay of these diseases. Using high-performance mass spectrometry-based proteomics, we explored the primary signaling pathways and liver proteome alterations triggered by hyperglycemia under the influence of a NETosis inhibitor.

Materials and Methods: Three groups of C57Bl/J6 mice were used: a) the control group with lower left ischemic limb made by excision of a part of the femoral artery and induced plantar wound (IR, n=5), b) the group with diabetes induced by streptozotocin and ischemic wounds (DIR, n=4) and c) the group DIRT (n=5) which received NETosis inhibitor treatment (Cl amidine, 10mg/kg b.w. for 2 weeks). The ischemic and wound procedures were performed after confirmed hyperglycemia (≥240 mg/dL). After 14 days the mice were euthanized and the liver was harvested for mass spectrometry-based proteomic analyses.

Results: The proteomic analysis revealed that proteins with significantly altered abundance are components of several metabolic pathways including Protein processing in endoplasmic reticulum, Biosynthesis of unsaturated fatty acids and Neutrophil degranulation. NETosis and neutrophils degranulation exhibited a significant increase of 7.5-fold in the protein abundance of S100A9 in the liver of diabetic animals. NETosis inhibitor induced a significant decrease by ~2-fold of the alarmin expression.

Conclusion: These preliminary results demonstrate an altered proteome of the diabetic liver tissue and a decrease in the abundance of S100A9 and COPB (coatomer subunit beta) proteins after NETosis inhibition treatment. Our study pursues the complicated relationship between NAFLD and DM2, allowing a better understanding of diabetic liver recovery under NETosis inhibitors.

Acknowledgement: The study was supported by the Romanian Academy and grants from the Ministry of Research, Innovation and Digitization (grant no. PNIIIP4PCE20211344 within PNCDI III).

CHROMOSOMES B: REGARDING TO PRESENCE AND EVOLUTION TO *ZEA MAYS*

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Even though the B (Cr.B) chromosomes named also supernumeraries-Cr., inert-Cr., selfish elements have been identified and are known for over 100 years, they have remained the subject of active study to the present day.

Who is Cr.B? In looking for a consensus definition, Camacho and Parker (1994) proposed: ***B chromosomes are additional dispensable chromosomes that are present in some individuals from some populations in some species, which have probably arisen from the A chromosomes but follow their own evolutionary pathway.***

Our investigations on Zea mays inbred lines (different generations from I1 to I5) originating from three maize landraces: Giulvaz, Gottlob and Recas revealed the presence and a specific behavior of Cr.B to each "genotype".

Their localization: their presence were identified in all kingdoms: in the plants (~2.3% species), in the animals there are much less (~1.5% species) and at the Fungus only 0.014% of the known species and 0.00056% of the estimated ones. In our case the Bs were found in all types of maize cells: somatic and germ.

Their number was: 1–5 Bs/cell at Gottlob, 1-2 on Giulvaz and 1-3 for Recas landraces. The inbred lines I1 to I3 pointed out the highest frequency of Bs. In the progressive homozygosity of maize genotypes, translocations and deletions were observed, which could explain the presence of Bs. Neo-B could be the secondary product of Robertsonian translocations, which generate a metacentric chromosome by the fusion of two acrocentric A chromosomes. Recent data of molecular analysis in maize have highlighted the existence of many DNA repetitive sequences in the B chromosomes being shared with the A chromosomes (Alfenito and Birchler, 1993), suggesting an intraspecific origin for Bs (Stark et al., 1996). In the advanced generations of homozygosity (I4-I5), lines with Cr.B and "clean" lines without additional chromosomes have been separated. It seems that in the "processed genotypes" Cr.B loses their informational reserve quality, thus being eliminated. The more species will be analyzed, the more Cr.B will be better known and will surprise us more.

IMMUNE MODULATION OF T CELLS BY PLATELETS AND MICROVESICLES IN EXPERIMENTALLY INDUCED ATHEROSCLEROSIS

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Platelets and circulating microvesicles (MVs) are emerging as important contributors to the development of inflammatory processes in atherosclerosis. T lymphocytes are direct targets of the immunological signals received from the circulation and integrate the information to elaborate an adaptive response.

The aim of the study was to investigate in vitro the effects of circulating platelets and microvesicles isolated from healthy mice on the immunomodulatory capacity of T cells in an experimentally induced animal model of atherosclerosis.

Materials and Methods: Platelets, MVs and splenic T cells were isolated from healthy C57BL/6J mice (C group) and ApoE-/- mice fed a high-fat, high-cholesterol diet for 12 weeks (HFHC group). We evaluated platelet activation by assessing CD62-P on their surface and determined plasma concentrations of Annexin-V+ MVs using flow cytometry technique. T lymphocyte proliferation and differentiation were performed in the presence of platelets or MVs isolated from control mice. The activation markers CD25, CD69 and CD154 were identified by flow cytometry, while the cytokines released in the culture media were measured by ELISA.

Results: In our experimental animal model of atherosclerosis we demonstrated that: (i) circulating platelets exhibited elevated levels of CD62-P; (ii) platelet-derived MVs were increased in number and unveiled greater levels of Annexin V; (iii) T lymphocytes presented an elevated inflammatory profile (IFN-g, TNF-±, IL-10 and IL-17) compared to control mice. Furthermore, functional assays showed that platelets and MVs derived from control mice decreased the in vitro proliferation and differentiation of T cells from healthy and HFHC mice and induced the release of IFN-γ by T cells isolated from control mice. MVs of healthy-origin had a significant role in controlling the splenic T lymphocyte immune response during atherosclerosis, mainly by inhibiting CD8CD69 and reducing IL-17 release.

Conclusion: The data demonstrated that microvesicles, rather than platelets, were associated with a reduction in the pro-inflammatory and cytotoxic effector phenotypes of T cells linked to atherosclerosis.

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N1- INFLAMMATORY NEUTROPHILS INDUCE THE TRANSITION OF CARDIAC FIBROBLASTS TOWARD AN INFLAMMATORY PHENOTYPE

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Introduction: Cardiac fibroblasts display phenotypic plasticity, with distinct phenotypic profiles that may be responsible for the wide range of functions in infarcted and remodeled hearts. Even though the inflammatory component is demonstrated as dictating the evolution post-myocardial infarct (MI), the impact of inflammatory cells that infiltrate the infarcted area post-MI on the modulation of fibroblast phenotype remains poorly understood. In this context, we investigated the phenotypic modulation of fibroblasts by factors released by the two major neutrophil subtypes found post-myocardial infarction, N1-inflammatory and N2-anti-inflammatory neutrophils.

Materials and Methods: Fibroblasts were allowed to communicate with different neutrophil subtypes using a two-chamber Transwell system, where the human cardiac fibroblasts (CF) were grown in the lower chamber and the neutrophils were added to the insert. The fibroblast phenotype was assessed by evaluating the expression of different inflammatory, remodeling, and pro-fibrotic molecules using qPCR, Western Blot, and ELISA assay and functionality by xCELLigence Real-time analysis and wound healing assay. The results obtained in vitro were validated in vivo using a MI-mouse model, obtained by ligation of the left anterior descending artery.

Results: The data demonstrated that factors released by N1 induce a pro-inflammatory and matrix-degrading phenotype in fibroblast, delineated by overexpression of inflammatory cytokines, chemokines IL-1β, IL-6, MCP-1, MIP-1α, RANTES, and metalloproteases MMP-1 and MMP-9. Conversely, the expression of molecules associated with fibrosis, such as collagen-I and -III, TGF-β, α-SMA, and CCN2 was reduced in fibroblasts after co-culture with N1 neutrophils. These modifications were accompanied by increased phosphorylation of p38MAPK and p65 subunit of NF-KB transcription factor. In vivo data confirmed the in vitro data, showing that at 24h post-MI, the infarcted cardiac tissue expressed increased levels of inflammatory molecules. Conversely, after 7 days, when N2 neutrophils were present, the inflammatory molecules were dramatically decreased and pro-fibrotic molecules were induced in the injured left ventricle.

Conclusion: Our findings indicate that different neutrophil subtypes may be responsible for changes in fibroblast polarization in myocardial infarction, thus orchestrating the reparative process.

Acknowledgement: This work was supported by Romania's National Recovery and Resilience Plan, NextGenerationEU, PNRR-III-C9-2022-I8-93, HeartCure project, Financing Contract no. 760063/23.05.2023, and by the Romanian Academy.

Keywords: cardiac fibroblast phenotype, neutrophils, myocardial infarction, hydrogel

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THE ROLE OF PHYTOCHEMICALS IN ORAL CANCER

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Introduction: Oral cancer is a serious oncological condition, characterized by a low survival rate and late diagnosis. Risk factors, such as alcohol and tobacco consumption, viral infections, UV radiation and chronic inflammation are frequently involved in the etiology of this type of cancer. Common treatments such as surgery, radiotherapy and chemotherapy are often accompanied by adverse effects that can influence patients' quality of life, which is why it is necessary to approach innovative therapies that can reduce toxicity and improve their effectiveness.

Methods: This paper reviews the therapeutic potential of natural herbal products and their use as adjuvant therapies in the treatment of oral cancer from online sources in databases (PubMed, Web of Science, Google Scholar, Research Gate, Scopus, Elsevier).

Results: In this regard, phytochemicals have recently captured the attention of researchers in the field due to their high potential in modulating essential signaling pathways on tumor progression. Specifically, phytochemicals inhibit critical oncogenic pathways, such as induction of apoptosis and inhibition of cell migration. They may also show a beneficial role in stimulating antioxidant enzymes and supporting the body's immunity against the development of malignant cells. The primary pathways targeted by phytochemicals are the epidermal growth factor signaling (EGFR) pathway, the NF-κB pathway, the Wnt/β-catenin pathway, the JAK/STAT pathway, and the mTOR pathway. By targeting these pathways, phytochemicals can lead to a strategy to reduce the incidence of oral cancer risk by preventing or slowing down the carcinogenesis process, by reducing inflammation and protecting healthy cell structure.

Conclusion: Phytochemicals can offer new perspectives in supporting oncology therapies, both due to their therapeutic efficacy and limited toxicological profile, as well as due to their accessibility. However, advanced studies are needed to optimize their administration and efficacy in the treatment of oral cancer.

PHD STUDENTS' PRESENTATION SESSION

LESS IS MORE: REDUCED PANCREATIC B-CELL MASS REVEALS METABOLIC ADAPTATION

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Background: Diabetes affects millions worldwide and is characterized by progressive β-cell dysfunction and death, leading to impaired insulin secretion and glucose homeostasis. Understanding β-cell adaptation mechanisms under metabolic stress is crucial, particularly how the remaining β-cells compensate when functional β-cell mass is reduced.

Aim: To investigate the early adaptive mechanisms in remaining β-cells following partial ablation, during short-term obesogenic diet exposure focusing on metabolic and molecular responses.

Methodology: We used the NSG RIP-DTR mouse model, that expresses the human diphtheria toxin receptor (DTR) driven by the rat insulin 2 promoter (RIP) in an immunosuppressed **Background:** We ablated ~50% of β-cells through DT administration and exposed the mice to a high-fat diet (60% calories from fat, HFD) plus high glucose water (20%, HGW) for 4 weeks. Metabolic parameters were evaluated, and transcriptomic analysis of isolated islets were performed. Glycemia, insulin secretion, lipid profiles and glucose tolerance were monitored.

Results: Initial DT administration successfully reduced β-cell mass by ~50%, and upon HFD+HGW exposure, mice exhibited increased body weight, elevated blood glucose, and significant increases in lipid parameters by 4 weeks. Interestingly, while these mice showed impaired first-phase insulin response in GTT, they maintained lower insulin and C-peptide levels compared to HFD+HGW controls with intact β-cell mass, suggesting preserved insulin sensitivity despite the metabolic challenge. Transcriptome analysis revealed 1465 differentially expressed genes, with top enriched biological processes including chaperone-dependent protein folding and ER unfolded protein response (UPR).

Conclusion: Our findings reveal that partial pancreatic β-cell reduction leads to a unique early metabolic adaptation under obesogenic conditions, partly through activation of UPR pathways that enhance protein folding capacity. This compensatory mechanism could be therapeutically targeted for maintaining the β-cell function in diabetes treatment.

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CRISPR/DCAS9 LONG-TERM TRANSCRIPTIONAL ACTIVATION OF HEPATIC KEY PROTEINS TO IMPROVE HDL FUNCTION

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Introduction: One of the main causes of cardiovascular diseases is atherosclerosis, which continue to be a leading cause of death worldwide, despite of therapies developed. An anti-atherosclerotic approach is to improve the level and function of high-density lipoproteins (HDLs). Liver is the major source of HDLs, and two of their key proteins are apolipoprotein AI (ApoAI) and paraoxonase 1 (PON1). Thus, we aimed to induce overexpression of the endogenous apoAI and PON1, and to maintain their activation for long-term in cultured human hepatocytes (Hep).

Materials and Methods: Human Hep (Huh7 line) were transfected with CRISPR/dCas9 plasmids for ApoAI or PON1activation, and control plasmids as reference. Gene expression of the target proteins was measured in cell lysates: (i) at 4, 7, and 14 days after transfection in the absence of selection antibiotics, and (ii) at 28 and 42 days after transfection, subsequent to the selection with antibiotics puromycin, blasticidin, and hygromycin B.

Results: Transcriptional activation of ApoAI in Hep using CRISPR/dCas9 system upregulated its gene expression 2 times at 4 and 7 days after transfection, compared to cells transfected with control plasmids (Cp). PON1 gene was overexpressed in Hep 6 times at 4 days and 2 times at 7 days after transfection versus Cp. The increased gene expression of ApoAI and PON1 diminished over time in the absence of selection antibiotics, and at 14 days after transfection it was no longer increased versus Cp. ApoAI gene expression in Hep exposed to selection antibiotics increased 9 times at 28 days and 7 times at 42 days versus Cp. PON1 gene expression in Hep exposed to selection antibiotics increased 19 times at 28 days and 15 times at 42 days after transfection versus Cp.

Conclusion: Long-term transcriptional activation of ApoAI and PON1 in human hepatocytes could be achieved by using CRISPR/dCas9 technology and selection antibiotics. Overexpressing ApoAI and PON1 in hepatocytes might be a promising tool to improve the level and function of HDL.

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IMPACT OF INCREASED DIETARY GLUCOSE INTAKE ON ADAPTIVE MECAHNISMS IN PANCREATIC β-CELLS

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Introduction: Dietary glucose intake triggers a metabolic stress response that increases the pancreas's insulin production requirements. As a response, healthy β-cells strategically transition through various functional states so that the insulin demand burden is overcame. However, these stress-induced adaptive responses may be dysfunctional in diabetes. Thus, we aimed to characterize how chronic, high-glucose exposure affects β-cell function, survival, and resistance to autoimmune destruction in a murine model of diabetes.

Methodology: Our experimental approach included 4 weeks (4w) old non-obese diabetic female mice (NOD) that were exposed to high-glucose water (HGW), or normal water (NW), for different periods of time. The pancreases and subsequently, the islets from each experimental group, were collected and analyzed by fluorescence microscopy and bulk RNAseq, respectively.

Results: Prolonged exposure (24w) of NOD mice to HGW resulted in a significantly decreased diabetes incidence. At shorter periods of HGW intake (8w), between the 3rd and 4th week, although the glycemia in the HGW group was higher than in the NW group, the glucose control was maintained. By complex immunofluorescence analysis, after 3 and 4w on HGW, we found differential levels of expression for proinsulin and insulin, defining several subpopulations of β-cells. Our results suggest an increased proinsulin-to-insulin processing in HGW group, compared to the control. Moreover, at 8w (4w HGW), insulin homeostasis was not affected, despite the immune stress present in NOD mice, combined with the additional glucose intake, suggesting adaptive responses. To investigate these molecular mechanisms, we conducted transcriptomic analysis of the islets from 3 and 4w HGW and NW. Gene Ontology (GO) enrichment analysis revealed that the metabolic pathways (fatty acids transport, amino-acid, lipolysis, cholesterol biosynthesis) were mostly dysregulated in HGW vs. NW group at 3 and 4w, respectively.

Conclusion: Our results suggest that pancreatic β-cells maintain their function under sustained stress through an adaptive mechanism that may include activation of metabolic pathways that provide the energy necessary for increased processing and secretion of insulin.

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BRAIN GLYCOSYLATION AND PARKINSON'S DISEASE DEVELOPMENT - MECHANISMS AND PROSPECTS

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Introduction: The growing incidence of both Parkinson's disease (PD) and cardiometabolic diseases, especially type 2 diabetes, is undeniable. Moreover, there is a correlation of 40% between the two conditions . We estimate a key role of dopamine in the glycosylation processes in the etiopathology of PD. The experimental model was achieved by MPTP-induced dopaminergic lesion.

Materials and Methods: The experimental part was carried out with CD21 mice randomly divided (n = 13) into MPTP and Control groups. MPTP was administered at 25 mg/kg b.w. sequential (1 dose each 2 days) and daily for 7 days for all MPTP mice. The whole brain was dissected out and cortical and striatal areas were prepared first of all for immunohistochemical assay for α-synuclein and GFAP, whereas tissue homogenates were subjected to ELISA α-synuclein and dopamine (DA) assay, gel electrophoresis in both native and denaturing systems, treated with periodic acid-Schiff reagents and silver stain protocol, and finally untargeted metabolomic analysis. Experimental data was also evaluated for the correlation coefficients and PCA using the PAST software.

Results: This research shows that the protein-free carbohydrate level significantly decreases in the MPTP-treated cortical homogenate, as seen on the Schiff stain. As for the striatum homogenates, the slight increase of protein-free carbohydrates in MPTP when compared to control is backed up by the metabolomic studies, which show increasing levels of free carbohydrates in MPTP-treated striatal homogenates when compared to control. The semi-quantitative analysis of glycoproteins, provided by the silver stain, highlights at 15-20 kDa a weaker signal in the MPTP striatal homogenate as opposed to control in the native gel electrophoresis. However, the opposite occurs in SDS-PAGE, as the signal increases in the MPTP striatal homogenate when compared to the control.

Conclusion: The results lead us to the conclusion that the PD pathology involves most probably a tetrameric or a pentameric glycoproteic complex, of which the glycosylation is deprived (either slowed down or prevented). SDS-PAGE and metabolomic studies highlight the role of fundamental metabolic pathways of carbohydrates, such as protein glycosylation and protein-free carbohydrates. In the light of these findings, PD has also a significant impact on the cortex, which is less expected than the one on the striatum, a conclusion which suggests that further studies need to be implemented.

TEHNOLOGII EEG: DE LA ACHIZIȚIA SEMNALELOR LA CONTROLUL FUNCȚIONAL

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Proiectul dezvoltă un sistem de monitorizare și interpretare a semnalelor EEG utilizând plăcuțele de dezvoltare STM32 nucleo, Arduino și EEG Click completat de o placă de extensie Mikroe pentru îmbunătățirea funcționalităților hardware. Mecanismul urmărește să convertească semnalele neurologice în comenzi digitale, având scopul de a traduce activitatea neuronală într-o formă procesabilă de către un sistem computerizat. Sistemul software utilizează o arhitectură modulară, concepută pentru achiziția, prelucrarea și analiza semnalelor EEG (electroencefalografice). Acesta implementează un pipeline complex, care include etape de filtrare digitală, segmentare temporală și extragere de caracteristici, esențiale pentru obținerea unor rezultate precise.

În cadrul procesului, algoritmii avansați, incluzând rețele neuronale convoluționale (CNN) și rețele neuronale recurente (RNN/LSTM), sunt utilizați pentru identificarea tiparelor complexe din semnalele EEG, precum potențialele evocate P300. Aceste modele sunt optimizate printr-un proces de antrenare intensă pe seturi de date EEG, îmbunătățind astfel capacitatea rețelei de a detecta și clasifica semnalele de interes cu o precizie mai mare.

Prin acest design sofisticat, sistemul software poate să analizeze semnalele EEG într-un mod eficient și adaptabil, avansând în cercetarea și aplicațiile neurotehnologice.

Inspirați de utilitatea acestei inovații, echipamentul este construit pogresiv, de la achiziția semnalelor de pe un număr redus de canale și prelucrarea lor cu acuratețe, până la achiziția dintr-un număr mare de canale folosind electrozi uscați conectați wi-fi și plasarea lor sub forma unei căști ergonomice pe individ. Impactul proiectului se reflectă în crearea unui sistem accesibil și eficient pentru persoanele cu deficiențe motorii, posibilitatea diagnosticării timpurii a afecțiunilor neurodegenerative, testare și diagnosticare persoane cu deficiențe psiho-cognitive.

METALLOPROTEINASES AND TISSUE REMODELING IN CARDIAC TRANSPLANTATION

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Cardiac transplantation is nowadays in a continuous progress due to the understanding of the cellular rejection mechanism. Matrix metalloproteinases (MMP) have been shown to be involved in the development of rejection.

Metalloproteinases are a family of 25 proteolytic enzymes (zinc-dependent endopeptidases) present in the extracellular matrix whose known role is to degrade its structural components.

Matrix metalloproteinases (MMPs) are part of the metzicin family, a superfamily of zinc-dependent endopeptidase present in the extracellular matrix, together with ADAMs (a-disintegrins and metalloproteinases) and ADAMTS (a-disintegrin and metalloproteinase with thrombospondin motifs) .

Based on the affinity for the structural domain of extracellular matrix components and subcellular distribution, MMPs were divided into membrane-bound MMP-MT-MMPs, namely collagenases, gelatinases, stromelysins and matrilysins.

Of these, particular attention is focused on gelatinases, especially MMP2, MMP9, as their contribution in cardiovascular pathology is known.

It has been shown that in the pathology of cardiac rejection, the influx and persistence of elevated levels of CD3 LT and CD68-positive macrophages aggravates the stage of acute cellular graft rejection and, implicitly, the increased degree of rejection is associated with elevated levels of proinflammatory cytokines (IL-6, TNF alpha, TGF beta) and elevated levels of MMP9, but not TIMP-1, which has an anti-inflammatory role. Thus, MMP9 has been shown to be responsible for the influx of LT and monocytes into the transplanted heart and is considered a marker for the inflammatory response in cardiac rejection. MMP9 activity has been found to increase directly proportional to the degree of rejection, with peak levels of MMP9 expression occurring in advanced stages of rejection, and in the future could be considered an important parameter to be included in standard criteria for the assessment and diagnosis of rejection grade.

Another important parameter to follow in the rejection phenomenon is the fibrosis process. The increased expression of MMP2 and MMP9, as well as TIMP1 and TIMP2, correlates with an increased expression of collagen types II and III, which in turn progressively increase with the degree of graft rejection. TIMP1 and TIMP2 activate fibroblast proliferation in the extracellular matrix and regulate collagen synthesis and production in the cardiac graft.

The domain of cardiac transplantation is an intriguing topic in the literature with regards to the phenomenon of rejection, and the analysis of the impact of different types of MMPs on cardiac remodeling and the study of factors influencing the activity of these proteases is ever more important. Understanding these mechanisms allows the highlighting of potential changes in post-transplant myocardial structure induced by the activity of these MMPs, in order to improve post-transplant pharmacological therapy and quality of life of these patients.

IMPACT OF CURCUMIN-LOADED EXTRACELLULAR VESICLES AND ANTI-PD-L1 ANTIBODIES ON A 3D MELANOMA MICROENVIRONMENT

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Melanoma is currently one of the most aggressive types of cancer due to it's capacity to invade and metastases surrounding tissues. Classical therapies have limited success, having to counter the great side effects. Promising results have been achieved using therapies based on immune checkpoint inhibitors, due to their immunostimulatory effects. Nonetheless, melanoma cells can become resistant to these treatments, highlighting the necessity for new therapeutic approaches to overcome this issue. In this study we aimed to develop a new melanoma therapy with curcumin-loaded extracellular vesicles (EVs-CURC) derived from Trp-2/CpG-ODN (Tyrosinase Related protein-2/Cytidine monophosphate guanosine oligodeoxynucleotides) pulsed dendritic cells, in combination with an antibody that functions as a PD-L1 immune checkpoint inhibitor, to enhance the immune response against melanoma cells. The efficacy of this combined therapy was evaluated using a melanoma spheroid model that replicates the immune environment of melanoma.

Extracellular vesicles derived from dendritic cells (DC2.4) activated with CpG-ODNs and pulsed with TRP-2 peptide, were purified by UF-SEC (Ultrafiltration-Size Exclusion Chromatography) and validated through DLS and western blot for specific exosome markers. CURC was passively loaded into EVs. To test the efficacy of extracellular vesicle with CURC, anti-PD-L1 antibodies were concomitantly delivered with the EVs-CURC, to a spheroid model comprising murine B16.F10 melanoma cells, DC2.4 dendritic cells and $CD8^+$ lymphocytes embedded in 1% commercial ECM. As a result, we obtained a 50% reduction in cell viability within the spheroids compared to control after a 48 h exposure ($p < 0.0001$) followed by a 2-fold decrease in total volume of the spheroid ($p < 0.01$). The uptake of the curcumin incorporated in EVs, was 3-fold higher compared to that of free CURC. Taking advantage of drug delivery capacity of extracellular vesicles and the immunomodulatory effect of curcumin, this nano-formulation can be used as a successful adjuvant to the already existing anti-PD-L1 immunotherapy to fight melanoma.

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BIOCHEMICAL MARKERS IN CHRONIC INFLAMMATORY BOWEL DISEASE IN PEDIATRIC PATIENTS

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Introduction: Chronic inflammatory bowel diseases (CIBD) are chronic disorders of the gastrointestinal tract of unknown cause, including ulcerative colitis, Crohn's disease, celiac disease (CD), and indeterminate colitis. In the present study, we aimed to analyse the impact of hypovitaminosis D on concentration changes for phosphorus, calcium, and magnesium in a group of pediatric patients with CIBD diagnoses offered by INMC specialists.

Materials and Methods: Subjects met the diagnostic criteria for CD, and informed consent was obtained (INMC-decision no.14119). Laboratory tests included haematological parameters (PLT, MPV, and PDW), Vit D, Ca, Mg, P, and expression of Ac-ATG-IgA. Biostatistical analysis was performed by comparing the CD and CIBD patients.

Results: The study included 223 pediatric patients (135 female and 88 male), aged 3-18 years. Analysis of Ac-ATG-IgA expression showed that out of total patients, 43.04% (n=96) had higher than normal values (0-20 U/ml), and for 7.17% (n=16), it was not determined. 96 patients have abnormal immune reactions to gluten and tissue transglutaminase, which may indicate the presence of CD and the degree of inflammation in the small intestine; the rest, even if they were diagnosed with CD, in fact, have CIBD. According to Ac-ATG-IgA expression, patients were classified into CD and CIBD groups, respectively. Vit D supplements take 81.44% of CD patients and 67.71% of CIBD patients. The heatmap plot showed that: i) there was a moderate positive correlation (0.52) between vitamin D level and vitamin D supplementation; ii) serum minerals showed moderate positive correlations between each other, suggesting their interdependence. There are no significant changes between vitamin D and mineral levels in either age group. Analysis of correlations between biochemical parameters within the same age categories indicated that there are significant negative correlations between vit D, Mg and P in the CD group of patients.

Conclusions: Evaluating Ac-ATG-IgA expression is important to differentiate CD patients from CIBD patients. Monitoring vit D levels by age groups helps assess mineral metabolism to guide nutritional management and adjust the supplements needed for each group of patients.

NONPROTEOLYTIC ACTIVATION OF METALLOPROTEINASES BY NITRO-OXIDATIVE STRESS PROMOTING MOLECULES

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Introduction: Matrix metalloproteinases (MMPs) are a family of zinc-dependent extracellular matrix (ECM) remodeling endopeptidases which have the function to degrade almost every component of the ECM. The aim of the research is to investigate the role of 3 molecules associated with nitro-oxidative stress, such as nitrite, peroxide and peroxynitrite, in the activation mechanism of metalloproteinases 2 and 9 respectively. MMPs exist in the inactive form of pro-enzymes and in the activated form, through the proteolytic or nonproteolytic pathway. Literature data, but also previous data obtained by us, suggested the key role of reactive nitrogen species in the nonproteolytic activation of MMPs.

Materials and Methods: In order to investigate the modulatory action on MMPs of the above-mentioned molecules, the zymographic method was used to highlight the catalytic activity of MMP2 and MMP9 in the absence and presence of nitrite, peroxide and peroxynitrite respectively. In parallel with the zymographic analysis, an electrophoretic migration staining with Comassie Brilliant Blue was performed. Data were integrated using Image J software and electrophoregrams were compared, with significant differences set at a p value of < 0.05.

Results: Examination of the zymography showed that in the presence of nitrite there was a complete activation of MMP2 while peroxide or peroxynitrite did not lead to this result. Normally, we would expect peroxynitrite to be the most potent activator of MMP2 but it is possible that the concentrations of the nitro-oxidative stress promoting molecules were either too high or too low, so that its rapid generation equaled its degradation. Thus, the stabilisation of vascular-induced nitric oxide (NO) as nitrite led to the total activation of MMP2.

Conclusion: The results lead us to the conclusion that the reactive nitrogen species (RNS), as well as reactive oxygen species (ROS) play a key role in the nonproteolytic activation of MMP2. Based on these findings, in a clinical contex, vasodilator therapy with nitrates, nitrosative stress generated in various toxicoses (e.g. hydrazines, NSAIDs), the evolution of atherosclerosis, hemolytic anemia syndromes when free Hb reacts with NO and generates reactive species of N are future zones of interest.

Acknowledgement: This research received support from a GTC grant awarded by Babes-Bolyai University (Grant No. 32939/22.06.2023) and AOSR-TEAMS-III 2024-2025 grant.

STUDIES ON THE ANATOMICAL STRUCTURE, ULTRASTRUCTURE, AND POLYPHENOLIC PROFILE OF *OENOTHERA BIENNIS* L. IN RELATION TO DIFFERENT PEDOCLIMATIC CONDITIONS

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Oenothera biennis L. is a plant species utilized in traditional medicine as well as in skincare products, pharmaceuticals, and the food sector. Although in North America, where it is endemic, it was originally used for its vegetative organs, these have remained largely unstudied after the introduction to Europe and Western Asia, where the seeds became the main part used and researched. Since the anatomy, phytocomposition, and biological activity may undergo changes under the action of environmental conditions, we aimed to comparatively study the structure and ultrastructure of vegetative organs, as well as the polyphenolic profile of O. biennis, in the context of two different pedoclimatic conditions belonging to the Western Plain and Eastern Carpathians, respectively. Climatic maps were obtained using GIS technology and data from 8 meteorological stations near harvesting sites. Anatomical analysis of vegetative organs was performed by light microscopy, and electron microscopy (SEM and TEM), while chemical analysis of whole plant was done with LC/MS. The results were analyzed in relation to the pedoclimatic data corresponding to sample collection sites. The results show significant anatomical differences as well as possible areas of synthesis and storage of bioactive compounds and may suggest a beneficial effect of higher mean annual temperatures and low cumulative precipitation on the polyphenolic profile of this species. This is the first report on the ultrastructure of the vegetative organs of the O. biennis species and the first comparative study simultaneously illustrating its geographical location, phenolic profile, and histology.

PHD STUDENTS' POSTER SESSION 2

TESTING THE ANTITUMOR POTENTIAL OF IONIC LIQUIDS WITH AMMONIUM GROUP

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Introduction: Ionic liquids (IL) have specific properties and characteristics that make them suitable for different applications, such as low production costs, easier synthesis, controllable polymorphism, functional integrity and environmental friendliness. One of the specific key characteristics of these substances is the incorporation of biologically active ions, which expands the potential biopharmaceutical applications, as antimicrobial, antioxidant, anti-inflammatory, antiproliferative and antitumor agents. The appropriate selection of the cation and the anion in the structure of the ionic liquid will generate specific physicochemical and biological properties, adapted for selective application.

Material and Method: A number of 4 ionic liquids: tetrabutylammonium chloride, tetrabutylammonium bromide, tetrahexylammonium chloride, tetrahexylammonium bromide were tested to highlight their antitumor potential. The evaluation of the antiproliferative potential of IL was done on 4 cell lines: MSC (mesenchymal stem cells), A375 (malignant melanoma cell line), HCT119 (colorectal carcinoma cell line) and CaCo2 (human colon adenocarcinoma). The cells were incubated with the experimental solutions for 24h, 48h, and 72h. Evidence of cytotoxicity was obtained by analyzing cell proliferation by the Vybrant cell proliferation assay, which is based on cell reaction with MTT.

Results and Conslusions: Tetrabutylammonium chloride, tetrabutylammonium bromide, tetrahexylammonium chloride and tetrahexylammonium bromide did not exert cytotoxic effects on the MSC line. The exposure of the cell lines to the tested liquids was dependent on the dose and the time of application, with the exception of the cells of the A375 line incubated for 24h with tetrabutylammonium chloride, where a hormesis effect was identified. ILs with chlorine in the molecule produced lower cytotoxic effects, in all cell lines studied, compared to ILs with bromide anion.

THE IMPORTANCE OF MATRIX METALLOPROTEINASE-9 (MMP-9) FOR MOTHERS AND NEWBORNS IN THE EARLY POSTPARTUM

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Introduction: Matrix metalloproteinase-9 (MMP-9), located on chromosome 20, is an enzyme class of the zinc-metalloproteinase family that degrades the extracellular matrix under physiological and pathological conditions. Peripartum (when women may have various conditions), the roles of MMP-9 vary: they intervene in tissue remodeling, critical role in pregnancy, birth and postpartum uterine involution, possible role in inflammation, permeability of the blood-brain barrier, depressive syndrome, etc. (potential biomarker). During the neonatal period, rapid, sometimes critical, transformations occur. and the roles of MMP-9 in newborns are diverse: one genotypic variant has been associated with higher birth weight and another, with lower birth weight, have critical roles in normal and pathological neurodevelopment.

Material and Method: In the cross-sectional study of the 40 mother-newborn pairs hospitalized at birth in the neonatology clinic of the 'Pius Brînzeu' County Emergency Clinical Hospital in Timisoara, in July 2022, various analyzes were performed (maternal MMP-9, cortisol, etc.). Statistical analysis, performed with R and GraphPad Prism 10 software, included descriptive statistics, correlation and regression.

Results: The average maternal age is 30 years and all had a normal delivery. On average, the babies' birth weight was 3274.25 g (2340-4440 g), gestational age 38.48 weeks (35-42 weeks) and APGAR/1st minute score, 9 (6-10). On the first postpartum day, the mean blood MMP-0 level was M = 144.6 (standard deviation SD = 33.68) ng/ml (reference value in Europe: 14.3-34.6 ng/ml). A significant correlation ($r = -0.398$, $p < 0.05$) was identified between MMP-9 level and cortisol. The relationship between MMP-9 level and absorbance was $R2 = 0.99$ ($p < 0.05$). Linear regression indicated that MMP-9 predicts cortisol, and 16.1% of the variability of cortisol is explained by MMP-9 level (determination coefficient $R2 = 0.16$).

Conclusions: 1) In mothers, the MMP-9 level and cortisol are significantly correlated, the MMP-9 level being a predictor for early maternal cortisol after birth. 2) The level of MMP-9, as a biomarker, can lead to improved diagnosis and care for mothers and newborns.

APOPTOTIC POTENTIAL OF TARGETED APO-1 LIGAND

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Introduction: Apo-1 ligand (Fas ligand) is a transmembrane protein expressed on several cell types such as cytotoxic T cells, natural killer cells, and endothelial cells. Once bound to its cognate receptor, the Apo-1 ligand triggers a cascade of intracellular events leading to apoptosis. The present work aims to demonstrate the apoptotic potential of a recombinant protein constructed from the following components: a signal peptide for secretion, a peptide with affinity for an adhesion protein, a trimerization domain, and the extracellular region of the Apo-1 ligand.

Materials and Methods: HEK-293 cells were transduced using a lentivirus to obtain stable clones secreting the recombinant protein. Protein expression was verified by immunofluorescence and Western Blot. Recombinant proteins were purified using ammonium sulfate precipitation and Nickel-beads batch chromatography. The recombinant protein concentration was tested by ELISA. HTB-14 cells were incubated with culture media from protein-secreting clones or purified proteins. Cell proliferation and viability were measured by the xCELLigence system and XTT assay. Cleaved caspase 3 in HTB-14 cells incubated for 48h with media containing recombinant proteins was detected by immunofluorescence, luminescence assay, and Western Blot.

Results: xCELLigence results showed that the rate of HTB-14 cell proliferation was significantly lower when the cells were incubated with the proteins of interest compared to the control group. In cells incubated with the recombinant protein containing the adhesion domain, caspase 3/7 activity was significantly higher compared to negative controls. A higher amount of cleaved caspase 3 was present after incubation with recombinant proteins containing the adhesion domain, as determined by Western Blot.

Conclusions: The apoptotic inducer Apo-1 ligand-induced HTB-14 cell death, alone as well as included in the mentioned protein constructs. The presence of the adhesion domain enhances apoptosis of the Apo-1 ligand.

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FUTURE DIRECTION IN CARDIAC ALLOGRAFT REJECTION. QUO VADIS?

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Rejection remains a major complication affecting long-term outcomes in heart transplant (HT) recipients. This work aims to provide a comprehensive overview of the current state and future direction in HT rejection. At the present time, the monitoring of heart allograft rejection is done by endomyocardial biopsy (EMB) wich is an invasive diagnostic procedure, involving the extraction of myocardial tissue from the right ventricular septum under fluoroscopic guidance, thus providing the necessary material for histopathological, immunohistochemical, or molecular analysis, offering critical insights into cardiac allograft rejection. Rejection is classified into three types—hyperacute, acute, and chronic—each identified through EMB histopathology and emerging diagnostic methods such as donor-specific antibodies (DSA), donor-derived cell-free DNA (ddcfDNA), and gene expression profiling, with the latter providing a high negative predictive value. DSAs are detected using solid-phase assays with HLA antigen-coated beads, where fluorescence indicates the presence and level of specific HLA antibodies. In 2018, the ISHLT included DSA monitoring in post-transplant guideline due to DSAs' links to antibody-mediated rejection (AMR), cardiac allograft vasculopathy (CAV), and graft dysfunction. Donor-derived cell-free DNA (ddcfDNA) is released into the recipient's bloodstream when the allograft's cells are damaged. After surgery, ddcfDNA levels initially increase but typically decrease and stabilizes at a basal level within 28 days or stabilize within a week, according to some studies. Elevated ddcfDNA levels are associated with episodes of acute rejection and tend to decrease following treatment, making ddcfDNA a reliable marker with a high negative predictive value for monitoring rejection in transplant patients. The future of heart transplantation may advance through various innovations, including gene editing and cell therapy, next-generation immunosuppressive agents, personalized immunosuppression protocols, nanotechnology and drug delivery systems, as well as tissue engineering and bioartificial hearts.

If the genes responsible for immune tolerance or immune activation can be edited so as to obtain a more favorable environment for the allograft, the doses of immunosuppressive medication would decrease, thus avoiding side effects.

Over 50 years since its introduction, EMB remains the "gold standard" for rejection monitoring in HT recipients, but other less invasive, complementary methods can reduce the number of necessary EMB.

THE ROLE OF POSTOPERATIVE DRUG TREATMENT OF HEART TRANSPLANTED PATIENTS IN REDUCING THE RISK OF ACUTE GRAFT REJECTION

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Introduction: Acute graft rejection (AGR) remains a major cause of morbidity and mortality in heart transplanted patients, despite effective immunosuppressive strategies. The objective of our study was to investigate the impact of postoperative drug therapies in relationship with the risk of 2-year AGR.

Material and Methods: Between January 2011 and December 2023, a total of 51 patients underwent cardiac transplantation in the Emergency Institute for Cardiovascular Diseases and Transplantation of Targu Mureș, Romania. At least two endomyocardial biopsies over a 2-year period, together with the details postoperative drug treatment details represented inclusion criteria. After applying the inclusion criteria, 36 patients fitted the study design.

Results: The early initiation of spironolactone was negatively correlated with the 2-year AGR (r=-0.41; 95%CI: -0,65 – -0.09, p=0.012), but without an impact on the 6-month mortality (r=0.55; 95%CI: -0,42 – 0.23, p=0.559).

Conclusion: The initiation of spironolactone early after heart transplant was correlated with a lower incidence of 2-year AGR. The anti-inflammatory cellular role of spironolactone might have an impact on cardiac transplanted patients regarding the occurrence of AGR.

Keywords: heart transplant; acute graft rejection; spironolactone; mortality

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THE EFFECTS OF PLANT DERIVED ARTEMISININS ON LEVELS AND POST-TRANSLATIONAL MODIFICATIONS OF INHIBITORY SYNAPSE PROTEINS AND MARKERS OF ADULT HIPPOCAMPAL NEUROGENESIS IN THE BRAIN OF AD-MICE

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Plant derived multi-target compounds represent encouraging therapeutic approaches for multifactorial diseases, such as Alzheimer's disease (AD). Disrupted balance between excitatory and inhibitory neuronal activity is considered a main driver of AD pathogenesis contributing to cognitive deficits. We studied the protein expression of $GABA_A$- and glycine receptor subunits as well as the protein level and phosphorylation of the inhibitory receptor anchoring protein gephyrin in a mouse model of AD and how treatment with artemisinins modulates these features. In hippocampal subregions of 12-months-old double transgenic APP/PS1 mice artemisinins increased the protein levels of both gephyrin, and of γ2-$GABA_A$-receptor subunits which are otherwise all reduced in the hippocampus of transgene mice compared to age matched wild type animals. Similar changes were detected for protein levels and number of glycine receptor α3 subunits. Whereas the increase of $GABA_A$ receptor protein in the hippocampus involved mainly synaptically localized receptors, as evidenced by confocal microscopy, in the case of glycine receptor α3 subunits predominantly the number of extrasynaptic localized receptors - thought to play a role in tonic inhibition- was elevated after treatment. Furthermore, we found, that the increased postsynaptic γ2-$GABA_AR$ subunit density coincide with an increased phosphorylation of gephyrin at S270 through the serine kinase cyclin kinase 5 (CDK5), both of which are increased in the 3-months-old mouse hippocampus upon artemisinin treatment. It seems that altered neurogenesis is also an early event in AD pathogenesis and its progressive decline in AD brains is correlated with disease progression. In our studies immunodetection of proliferation and cell-type specific biomarkers in artesunate treated AD-mice indicated specifically an increase of doublecortin expressing neuroblasts and immature neurons in the subgranular zone of the hippocampus already in early stage of the disease. Thus, our present studies reveal that in mouse models of AD the plant derived compounds, artemisinins modulate inhibitory synapse protein levels and hippocampal neurogenesis, two important factors of AD pathogenesis, probably to maintain or restore brain homeostasis.

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