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ΠΡΟΦΟΡΙΚΕΣ ΑΝΑΚΟΙΝΩΣΕΙΣ / ORAL PRESENTATIONS

Stressful and dietary interventions in high anxiety: what about mitochondrial turnover?

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Aim: Stress is a major risk factor for anxiety disorders and individuals with high anxiety are more vulnerable to stress effects. Additionally, altered dietary habits can lead to eating disorders and individuals suffering from eating disorders show concurrent anxiety-related symptomatology. Recent literature strongly supports that mitochondria-related pathways mediate stress responses, regulate dietary habits and shape anxiety phenotypes. However, whether stressful and dietary interventions in a high anxiety background affect the mitochondrial turnover system, i.e. the highly coordinated processes of mitochondrial biogenesis, fission, fusion and mitophagy, is largely unexplored.

Materials-Methods: Here, we investigated mitochondrial turnover changes induced by acute stress exposure or food limitation in a mouse model of high anxiety-related behavior (HAB). Male and female HAB mice were subjected to an acute restraint stress (ARS) or to a limited food access (LFA) protocol, respectively. Their anxiety and depression-like behavior was then evaluated by a behavioral test battery. mRNA and protein levels alterations related to the mitochondrial turnover machinery were explored in biologically relevant brain regions.

Results: Our results showed that LFA increased depression-like behavior in the female HAB mice. Both LFA and ARS protocols induced significant changes in the mRNA levels of key players involved in the mitochondrial turnover system. In the cingulate cortex of male HAB mice subjected to ARS, the mRNA levels of molecular markers implicated in biogenesis, fission, fusion and mitophagy were altered vs. the control group. Differential gene expression associated with fission and mitophagy was also observed in the hypothalamus of the female HAB mice subjected to the LFA protocol vs. the control group. Notably, the protein levels of the fusion mediator mitofusin 2, negatively correlated with the % weight loss in the LFA group.

Conclusions: Overall, our data show that mitochondrial turnover is implicated in responses to stress exposure and dietary interventions in a high anxiety background. Clarifying the neurobiological mechanisms linked with mitochondrial quality control will provide new therapeutic targets and candidate biomarkers to alleviate the symptomatology of people suffering from eating and stress-related disorders.

Familial Hypercholesterolemia: Is Apolipoprotein A2 the next therapeutic target?

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Familial Hypercholesterolemia (FH) is an inherited metabolic disorder, associated with high cardiovascular disease rick, mostly due to elevated LDL-C concentrations in plasma. Numerous clinical studies involving either homozygous or heterozygous FH patients show that this risk is also strongly correlated with increased plasma triglyceride levels and postprandial hypertriglyceridemia. The pathophysiology of postprandial hypertriglyceridemia has been related to several gene loci, involved in fat load response (apolipoproteins B, E, C3, A1, A4, CETP, lipoprotein lipase and FABP2), raising the possibility that other apolipoproteins might contribute to the development of secondary lipid disorders in FH patients.

Here we investigated the role of apolipoprotein A2 in triglyceride metabolism and adipose tissue metabolic activity in the ldlr knockout mouse model, using adenovirus-mediated gene transfer of human APOA2.

Our results show that the reduction of plasma triglyceride levels is accompanied by significant accumulation of triglycerides in the liver of the ldlr-/- mice, infected with AdAPOA2 two weeks post WTD feeding, compared to the control group, due to the increased de novo biogenesis of triglycerides and fatty acid storage in their liver. Moreover, the expression of human APOA2 in FH mice leads to the inactivation of white adipose, the most important fat storing tissue, forcing BAT to work towards ATP production. Taken together, our data suggests that apolipoprotein A2 could be considered as a therapeutic target for metabolic disorders related to FH.

Niclosamide attenuates fibrotic responses in subepithelial pulmonary myofibroblasts

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Aim: Our aim was to investigate the possible anti-fibrotic effect of Niclosamide on Subepithelial Lung Myofibroblasts (SELMs) after stimulation with pro-inflammatory and pro-fibrotic cytokines.

Materials & Methods: SELMs were isolated from tissue biopsies of patients undergoing surgery for lung cancer and then stimulated, in the presence or not of Niclosamide (30nM and 100nM), with: a) TNF-α and/or IL-1α and b) TGF-β1. Expression of Collagen type I and III, and Fibronectin was studied using qRT-PCR and total protein collagen production was measured using the Sircol Assay. Migration of SELMs was studied using the Wound Healing Assay.

Results: Niclosamide had no effect on baseline mRNA expression levels of Collagen type I, III or Fibronectin of unstimulated SELMs, while stimulation with TGF-β1, IL-1α and/or TNF-α upregulated this expression. Treatment with Niclosamide ameliorated the effect of almost all cytokine stimulations; Collagen type I (IL-1α: 0.52-fold, ±0.01, TNF-α: 0.74-fold, ±0.03, IL-1α+TNF-α: 0.76-fold, ±0.07, and TGF-β1:1.31-fold, ±0.16, $p<0.05$), type III (IL-1α: 0.75-fold, ±0.02, TNF-α: 1.07-fold, ±0.19, IL-1α+TNF-α: 0.81-fold, ±0.11 and TGF-β1:0.77-fold, ±0.31, $p<0.05$) and Fibronectin (IL-1α: 1.02-fold, ±0.08, TNF-α: 0.77-fold, ±0.03 and IL-1α+TNF-α: 1.11-fold, ±0.06, $p<0.05$). At the protein level, total production of collagen of TGF-β1-treated SELMs (115%, ±0.55, $p<0.0001$) was reduced to baseline after treatment with 100nM Niclosamide (104.5%, ±1.37, $p<0.001$). TGF-β1-treated SELMs' migration was increased (118.3%, ±8.8, $p<0.05$), while treatment with 100nM Niclosamide debilitated the migratory ability of both stimulated (68.02%, ±2.96, $p<0.0001$) and unstimulated SELMs (69.75%, ±4.24, $p<0.0001$).

Conclusions: In this study we showcase an anti-fibrotic effect of Niclosamide on SELMs stimulated with profibrotic and pro-inflammatory cytokines. These data suggest a possible therapeutic effect of Niclosamide in pulmonary fibrosis.

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Colonoid development from human epithelial crypts: an ex vivo model for studying intestinal inflammation

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Aim: To develop and characterize Colonoids from human epithelial crypts, and establish an in vitro model for studying intestinal inflammation.

Materials & Methods: Human epithelial crypts were isolated from endoscopic biopsies, treated with a commercially available kit and differentiated into Colonoids. Colonoids were stained against epithelial markers and characterized using immunofluorescence. Finally, Colonoids were stimulated with inflammatory mediators (5ng/ml IL-1α and 50ng/ml TNF-α) for 12 hours and the mRNA expression of pro-inflammatory chemokines, CXCL1, CXCL8, CXCL10, CXCL11, CCL2 and CCL20 was examined by reverse transcription quantitative PCR.

Results: Characterization revealed that Colonoids were successfully developed, as they consisted of E-cadherin-, Cytokeratin- and EPCAM-expressing epithelial cells, MUC2 goblet cells and Lgr5+ epithelial stem cells. Untreated Colonoids had a baseline mRNA expression of all the studied chemokines. Stimulation with IL-1α and TNF-α (2C) for 12h led to a statistically significant increase in the mRNA expression of all the studied chemokines, compared to unstimulated Colonoids: CXCL1 (13.07-fold ±2.52, $p<0.05$), CXCL8 (6.59-fold ±1.20, $p <0.05$), CXCL10 (60.34-fold ±7.57, $p<0.01$), CXCL11 (7.70-fold ±0.74, $p<0.01$), CCL2 (39.40-fold ±5.86, $p<0.01$) and CCL20 (534.80-fold ±41.73, $p<0.01$).

Conclusions: Our results suggest that Colonoids are functional and could be used both as an ex vivo model for studying the pathogenetic mechanisms of intestinal inflammation, and as a preclinical model for investigating the effects of new anti-inflammatory drugs.

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Immune Checkpoint Inhibitors Cardiotoxicity is mediated via early endothelial activation, increased autophagy and inflammation in the myocardium

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Aim: Immune checkpoint inhibitors (ICIs) are immunotherapeutics with profound anti-cancer efficacy and severe immune-related adverse effects (irAEs). ICI-induced cardiotoxicity, is among the most life-threatening irAEs with elusive pathomechanism. Herein, we investigated the cardiotoxic effects of ipilimumab (IPI, anti-CTLA-4), pembrolizumab (PEM, anti-PD-1) and avelumab (AVE, anti-PD-L1) in vitro and established an in vivo model of ICI-related cardiomyopathy.

Materials and Methods: Primary murine cardiomyocytes (mAVCs) and spleenocytes were isolated and underwent IPI, PEM and AVE treatment for 24h at concentrations from 0 to100 μg/ml. Their conditioned media were transferred onto mAVCs for 24h. Cellular viability was assessed by MTT. Human (hu-PD-1) and Murine PD-1 (muPD-1) were biotechnologically produced and PEM binding was assessed by circular dichroism (CD) and in silico. C57BL6/J male mice were randomized into i. Control (IgG4, 2mg/kg, ip) and ii. PEM (2mg/kg, ip) (n=9/group) groups and treated for 5 weeks. PEM dose was directly translated from humans. Mice underwent weekly echocardiography analysis and blood sampling, while at the endpoint, mice were sacrificed for blood and myocardial sampling, histology and immunochemical analyses. Since cardiotoxicity was evident at 2 weeks of administration, the in vivo experiments were repeated for 2 weeks (n=5/group).

Results: IPI was excluded from the study due to spleenocytes' cytotoxicity. Only PEM could induce dose- and Immune cell (IC)-dependent cytotoxicity, characterized by increased inflammatory markers in spleenocytes and inflammatory, autophagy and endoplasmic reticulum (ER) stress markers in the mAVCs. PEM binding on the muPD-1 was confirmed by CD and in silico. In vivo, PEM decreased Fractional Shortening% (FS%) after 2 weeks, an effect exacerbated after 5 weeks of treatment. Intramyocardial IC infiltration, myocardial fibers' atrophy and an increase in myocardial and circulatory Lys6Clow monocytes and in circulatory T-cells was observed in PEM group at 5 weeks. Molecular analysis revealed that PEM induces early endothelial activation at 2 weeks which leads to increased autophagy, ER stress and inflammation signaling after 5 weeks.

Conclusions: PEM induces IC-dependent cytotoxicity on mAVCs via inflammation, autophagy and ER-stress, whereas in vivo it induces cardiotoxicity via FS% decrease, early signs of endothelial activation and subsequent establishment of inflammation and autophagy in the myocardium.

The challenge in regulating clinical trials of Advanced Therapeutic Medicinal Products in Europe: a review of the Good Clinical Practice and Good Laboratory Practice guidelines

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Aim: Advanced Therapeutic Medicinal Products (ATMPs), a medicinal class including gene therapy, somatic cell therapy and tissue engineering, offer pioneer therapeutic approaches to rare and ultrarare diseases in the new era of precision medicine. Until now in EU/EEA 19 ATMPs have been granted marketing authorization but only 12 (brand names: Yescarta, Kymriah, Luxturna, Spherox, Alofisel, Strimvelis, Imlygic, Holoclar, Zolgensma, Abecma, Libmeldy, Tecartus) are still on the market, as their intrinsic complexity poses several challenges on their journey from basic research and development, until post-marketing monitoring. It is therefore necessary that ATMPs' clinical trials adjust to this complexity.
Materials and methods: This study represents an effort to review the Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) guidelines of ATMPs, stressing out the difficulties that encounter both researchers and competent authorities and their efforts to surpass them.
Results: Guidelines for ATMP's clinical trials, while complying with the Directive 2001/83/EC, need adaptations and implementation of additional measures, as the Regulation 1394/2007 mandates. GLP and GCP follow the Risk-Based Approach and the clinical trial design is amended regarding the risk-benefit ratio for the patients. Study population, comparators, placebo, dosing ranges, and blinding need a careful design tailored to the product's special characteristics and due to the lack of quality standards comparability and reproducibility are often hampered. Safety is also a major issue and commonly a long term follow up period is necessary to track the adverse effects of the product, together with a bidirectional traceability system of the trial's data and retention of its samples. All these challenges also raise difficulties in setting appropriate pricing and reimbursement systems.
Conclusions: Clinical trials of ATMPs have multidimensional regulatory problems, thus it is a challenge for the competent authorities, the researchers, and the healthcare professionals to collaborate more efficiently with better structured pipelines.

Oligodendrogenesis as a potential therapeutic target in Alzheimer's Disease

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Aim: Myelin breakdown represents an early stage event in Alzheimer's Disease (AD). However, it is still unclear whether myelin loss is attributed only to exogenous factors like inflammation or to impaired oligodendrogenesis as well. TrkB is a neurotrophin receptor with a well-known pro-myelinating role in the Central Nervous System (CNS). BDNF binds TrkB and enhances myelination by increasing the density of oligodendrocyte progenitor cells (OPCs). However, endogenous neurotrophins are characterized by short half-life and inability to access the blood-brain barrier (BBB). Here, we study the oligodendrocyte pathology in 5xFAD mice (animal model of AD) and examine the potential of two novel synthetic molecules that act as BDNF mimetics to up-regulate oligodendrocyte differentiation in physiological and AD conditions.

Materials and Methods: The number of OPCs and myelin levels were compared in wild type (wt) and 5xFAD mice with immunocytochemical analysis for PDGFRa+ and MBP, respectively. OPCs were isolated from wild type animals (p3) and were differentiated towards O4+ oligodendrocytes under physiological and AD related conditions (presence of Amyloid-β). During the differentiation process cells were exposed to two BDNF mimetic compounds (TC508 & TC509) in order to investigate their effect on cell differentiation capacity. Additionally, we studied the effect of TC508 & TC509 on adult hippocampal neural stem cell (NSCs) differentiation towards oligodendrocytes.

Results: Comparison of myelin levels in 12 months old wt and 5xFAD mice revealed a significant myelin loss in the hippocampal regions CA1 and CA3 of the dentate gyrus in 5xFAD mice. Immunohistochemical analysis for PDGFRa+ showed reduced number of OPCs in 5xFAD mice suggesting defective oligodendrogenesis. We targeted TrkB signaling in primary OPCs by using two synthetic BDNF mimetics, TC508 and TC509, which selectively activate the TrkB receptor. Our results indicate that both compounds highly promote primary OPCs differentiation to oligodendrocytes under both physiological and AD related conditions (presence of Amyloid-β). Furthermore, TC509 promotes adult NSC differentiation towards OPCs enabling earlier targeting of oligodendrocyte fate commitment.

Conclusion: Our study provides evidence that oligodendrogenesis represents an appealing target in AD. We introduce two novel BDNF-micromolecular mimetics as promising lead therapeutic agents in the field of myelin regeneration and restoration.

Investigation of Empagliflozin cardioprotective effects on distinct cell populations in vitro and in vivo

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Purpose: Empagliflozin (EMPA) is a sodium-glucose co-transporter 2 (SGLT-2) inhibitor and a drug approved for type 2 diabetes mellitus (T2DM) and heart failure independently of T2DM. The cardioprotective mechanism beyond its antidiabetic action remains elusive. We have shown that EMPA reduces infarct size in normoglycemic mice subjected to myocardial ischemia-reperfusion (IR) and protects endothelial cells (ECs) against hypoxia/reoxygenation (Hyp/Reo) injury. We aimed to determine EMPA's distinct effects on three cell populations: cardiac ECs, cardiac fibroblasts (FBs) and cardiomyocytes (CMs). Methods: Primary adult murine cardiomyocytes (pAMCMs) and primary cardiac fibroblasts (pFBs) were isolated from C57Bl6 murine male hearts. Cells were treated with EMPA (100-500nM) prior to 24h Hyp/1h Reo and cell death was evaluated. To investigate the mechanism of EMPA in vivo, C57Bl6 male mice were randomized into two groups: 1) Control receiving vehicle and 2) EMPA receiving EMPA 10mg/kg/day for 6 weeks which is a clinically relevant dose. Then, mice were subjected to 30'I/2hR, the heart was collected to isolate CMs. The non-CMs populations were stained with CD45, CD31 and CD90 markers and ECs and FBs were sorted. RNA was obtained from the cell populations and 3'mRNA sequencing was applied to investigate the pathways affected by EMPA treatment. An additional cohort of mice was employed to validate the results at mRNA level. Results: EMPA pre-treatment protects pFBs but not pAMCMs from Hyp/Reo injury. In vivo, EMPA treatment affects ECs with 211 genes being significantly altered (FDR<0.05). Pathway enrichment analysis in ECs revealed that EMPA pre-treatment significantly alters pathways related to extracellular matrix organization, matrix degradation and immune cell adhesion. EMPA does not change the FBs' gene expression profile. In CMs, EMPA affects the expression of 811 genes (FDR<0.1) and pathways related to mitochondrial metabolism. The expression levels of significant genes of our dataset including Mmp-2, Timp-1, Icam, Vecam, etc are validated in the independent mice cohort. Conclusions: EMPA protects pFBs against Hyp/Reo injury in vitro. EMPA pre-treatment in vivo mainly affects the transcriptome of ECs at late reperfusion and the protective mechanism involves the regulation of extracellular matrix and immune cell adhesion.

Association of MTHFR, MTR and MTRR gene polymorphisms with antipsychotic treatment response of psychotic patients in a naturalistic setting

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Aim: The enzymes, 5-10 methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS), and methionine synthase reductase (MSR) help channel folate metabolites into the main s-adenosylmethionine (SAM) pathway. In humans, they are encoded by the MTHFR, MTR and MTRR genes, respectively. SAM is a crucial coenzyme for the epigenetic regulation of gene expression and the enzymic activity of catechol-O-methyltransferase (COMT), a significant modulator of catecholamine availability in the prefrontal cortex. The aim of this study was to examine the association of common polymorphisms of MTHFR (rs1801133), MTR (rs1805087), and MTRR (rs1801394), with response to antipsychotic treatment of psychotic patients, in a naturalistic setting, in Greece.

Materials and Methods: One hundred and sixty Greek patients suffering from schizophrenia and other psychotic disorders were successively enrolled in this observational study. SNP genotyping was accomplished with established RLFP-PCR methods. The Positive and Negative Syndrome Scale (PANSS) was used as a measure of symptom intensity for positive, negative and general psychopathology symptoms, determined at presentation and after one month of treatment. The Calgary Depression Scale for Schizophrenia was used for assessing depressive symptoms. Serum folate and vitamin B12 concentrations were determined both before and following treatment.

Results: We have detected a significant association of rs1805087 with the difference in the positive symptom PANSS subscale values (AA vs. G carriers; $p < 0.001$, Mann-Whitney). An apparent association was also noted between the same polymorphism and the change in Calgary scale values (AA vs. G carriers; $p = 0.010$, Mann-Whitney). Patients with the AA genotype responded better in both cases. Patients' serum folate and B12 levels did not correlate with PANSS or Calgary scale changes.

Conclusions: The MTR polymorphism appeared to be a significant determinant of response to antipsychotic drugs with respect to positive symptoms and, perhaps, depression, in our study.

Dysregulation of Akt downstream signaling in peripheral blood mononuclear cells of drug-naïve, first-episode of psychosis patients

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Aim: Schizophrenia is characterized by a complex interplay between genetic and environmental risk factors converging on prominent signaling pathways that orchestrate brain development. The Akt/GSK3β/mTORC1 pathway has long been recognized as a point of convergence and etiological mechanism but despite evidence suggesting its hypofunction it is still not clear if this is already established during the first episode of psychosis (FEP).

Materials and Methods: Here, we performed a systematic phosphorylation analysis of Akt, GSK3β and S6, a mTORC1 downstream target, in fresh peripheral blood mononuclear cells (PBMCs) from drug-naïve FEP patients and control subjects.

Results: Our results suggest two distinct signaling endophenotypes in FEP patients. GSK3β hypofunction exhibits a promiscuous association with psychopathology, and it is normalized after treatment, while mTORC1 hypofunction represents a stable state.

Conclusions: Our study provides novel insight on the peripheral hypofunction of the Akt/GSK3β/mTORC1 pathway and highlights mTORC1 activity as a prominent integrator of altered peripheral immune and metabolic states in FEP patients.

Cannabidiol modulates impaired prefrontal glutamatergic function and oscillatory activity in experimental ketamine-induced schizophrenia

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Background and objectives: Repeated ketamine (KET) administration in subanesthetic doses has been used to generate schizophrenia-like behavioral features in rodents, while the neurobiological underpinnings are yet to be elucidated. Cannabidiol (CBD), a psychoactive but non-addictive cannabis compound is reported to present antipsychotic properties, but the mechanisms involved are poorly understood. This study aims to evaluate the impact of repeated KET administration on glutamate and GABA neurotransmission, on the brain's oscillatory activity and schizophrenia-associated behavior, while exploring the modulatory role of CBD.

Methods: Male adult Sprague-Dawley rats were treated with subanesthetic KET for 10 days, followed by a 5-day long treatment with 10 mg/kg/day of CBD. Subsequently, rats underwent a battery of behavioral tests consisting of the open field, object recognition task, social interaction, and amphetamine challenge in the open field. High Performance Liquid Chromatography (HPLC) provided estimates of glutamatergic and GABAergic neurotransmission, while glutamatergic signaling and neuroplasticity markers were quantified using Western immunoblotting in specific rat brain areas implicated in schizophrenia. Additionally, local field potentials (LFPs) were recorded in the medial prefrontal cortex simultaneously with the dorsomedial striatum or ventral hippocampus in sevoflurane-anesthetized rats.

Results: KET-treated rats displayed hyperlocomotion, impaired recognition memory, and social behavior, only when KET treatment was not followed by CBD administration. Neurochemical analyses revealed region-specific KET effects on glutamate and GABA tissue levels, that were modulated by CBD. LFP recording analysis revealed that KET affects the prefrontal power spectrum density of gamma-frequency band, and this effect is mitigated by cannabidiol.

Conclusion: Repeated KET administration induced a schizophrenia-related bio-phenotype in terms of behavior and the subsequent neurochemical and electrophysiological analyses. CBD ameliorated core behavioral aspects of this schizophrenia model, while neurochemical and neurophysiological findings revealed that KET mimics the neurophysiological abnormalities observed in the prefrontal cortex of patients with schizophrenia. Current findings further characterize the KET-induced schizophrenia model and enrich our understanding of the mechanisms underlying CBD's antipsychotic potential.

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The deubiquitinase activity of CYLD is required for B cell differentiation.

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Aim: CYLD is a functional deubiquitinase that regulates several signaling pathways, crucial for cell survival and apoptosis. Perturbations of CYLD expression and/or activity have been associated also with emergence of several forms of solid and hematological tumors. A number of studies imply that CYLD is involved in B cell differentiation and activation, however its exact role remains unclear. This study aimed at elucidating the role of CYLD in B cell lymphopoiesis, using transgenic animals and -omics technologies.
Materials and Methods: We generated mice with targeted truncation of the catalytic domain of CYLD since the early stages of B cells differentiation. These mice were characterized immunophenotypically by flow cytometry and immunohistochemistry and functionally by immunization with T-dependent and T-independent antigens. Single cell RNA sequencing (scRNA-seq) was performed to identify affected signaling pathways.
Results: The Mb1CreCyld(flx/flx) mice we generated do not express catalytically active CYLD from the Pro-B cell differentiation stage. These mice displayed a marked reduction in spleen size and cellularity, compared to control (CYLDflx/flx) littermates, while histopathology findings indicated marked differences in the architecture and organization of germinal centers. Immunophenotyping of peripheral blood showed that mature B cells were virtually exhausted in the peripheral blood and severely depleted in the spleen of Mb1CreCYLD(flx/flx) compared to control mice. In the bone marrow of Mb1CreCYLD(flx/flx) mice the proportion of Pro-B cells was elevated, whereas immature and mature B cells were diminished compared to control mice. From a functional standpoint, Mb1CreCYLD(flx/flx) mice failed to mount an immune response, when immunized with T-dependent or T-independent model antigens. Analysis of scRNA-seq data from bone marrow-B cells indicated that several key metabolic pathways, including oxidative phosphorylation, glycolysis and fatty acid metabolism were perturbed.
Conclusions: Our findings indicate that the deubiquitinase activity of CYLD is essential for anatomical and functional maturation of B cells. Interestingly, scRNA-seq data imply a link between CYLD and immunometabolism, which could lead to better understanding not only of CYLD biology and B cell lymphopoiesis but also of the pathogenesis of hematological malignancies with a perturbed metabolic profile.

A multi-omics bioinformatics analysis on the biological background of two Heart Failure subphenotypes, Dilated and Ischemic Cardiomyopathy

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Aim: Heart failure (HF) is a complex clinical syndrome with a prevalence of 1-2% of the population in developed countries, leading to high morbidity. Although HF pharmacotherapy has come a long way, current therapeutic approaches are relatively etiology agnostic and focus on symptom alleviation. In this study, we aimed to identify the gene expression and protein signature of the two subphenotypes of HF dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM), coined as DiSig and IsSig, respectively.

Materials and Methods: Omics data were accessed through GEO repository for transcriptomic and PRIDE repository for proteomic datasets. Sets of differentially expressed genes and proteins comprising DiSig and IsSig were analysed by a multilayered bioinformatics approach. Enrichment analysis via the Gene Ontology was performed through the Metascape platform to explore biological pathways. Protein-protein interaction networks were analysed via STRING API and Network Analyst, while multi-omics visualization was achieved through OmicsNet.

Results: Intersection of transcriptomic and proteomic analysis showed 10 differentially expressed genes/proteins in DiSig (AEBP1, CA3, HBA2, HBB, HSPA2, MYH6, SERPINA3, SOD3, THBS4, UCHL1) and 15 differentially expressed genes/proteins in IsSig (AEBP1, APOA1, BGN, CA3, CFH, COL14A1, HBA2, HBB, HSPA2, LTBP2, LUM, MFAP4, SOD3, THBS4, UCHL1), while 8 molecules are shared by both DiSig and IsSig. Common and distinct biological pathways were retrieved, allowing for their molecular characterization. Extracellular matrix organization, cellular response to stress and transforming growth factor-beta were common between two subphenotypes. Muscle tissue development was dysregulated solely in DiSig, while immune cells activation and migration in IsSig.

Conclusions: Our bioinformatics approach sheds light on the molecular background of HF etiopathology showing molecular similarities as well as distinct expression differences between DCM and ICM. DiSig and IsSig encompass an array of “cross-validated” molecules at both transcriptomic and proteomic level, which can serve as novel pharmacological targets and possible diagnostic biomarkers.

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Prenatal alcohol exposure induces long-term alterations in the juvenile rat striatum complicated by experimentally-induced status epilepticus

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Aim: Fetal Alcohol Syndrome (FAS) is a developmental disorder that arises from prenatal alcohol exposure (PAE). Children with FAS have -on average- smaller brain and malformations in the frontal lobe, cerebellum, hippocampus and basal ganglia leading to motor, mental and behavioral deficits. In addition, 12% of individuals with FAS experience one or more seizures throughout their lives and they develop epilepsy at a rate of 6% compared to the 1% of the general population. The aim of this study was to investigate the effect of one status epilepticus (SE) episode at two different developmental stages in the rat striatal nuclei caudate-putamen (CPu), nucleus accumbens core (AcbC) and shell (AcbSh), in the presence/absence of PAE.

Methods: We studied four groups of Sprague-Dawley rats; Control: naive animals, PAE: animals prenatally exposed to ethanol and up to two weeks after birth, PTZ: animals with a SE episode induced at PD21 (weaning), or PD60 using pentylenetrazole (PTZ, GABAA antagonist) and PAE-PTZ: PAE animals with a SE episode induced at PD21 or PD60. Animals were sacrificed 40 days after the SE, at the respective ages of 2 and 3.5 postnatal months. Cresyl staining was used for Nissl body detection and neuropathology evaluation, while NeuN immunoreactivity and DAPI staining were used for neuronal and total cell number assessment, respectively. Statistical analysis was performed using GraphPad Prism software.

Results: Striatal Nissl body density, total cell number and CPu neuronal number were reduced in older (3.5mo) vs younger (2mo) animals. Furthermore, Nissl body density and cell number in striatum (CPu/AcbC/AcbSh) were found reduced in 2mo old PAE (vs Control) animals; this effect was evident only in CPu at 3.5mo. A single SE at PD21 increased Nissl body density in PAE (vs Control) striatum; in CPu this increase was accompanied by a concomitant increase in cell number. Interestingly, no changes were detected in PAE animals with a single SE at PD60.

Conclusions: PAE disturbs the physiological developmental changes in striatum, possibly leading to neuronal deficits. The occurrence of a SE in PAE animals affects these changes age-dependently, suggesting that early seizures may complicate the PAE effects in striatal function.

CTH/MPST double ablation results in enhanced vasorelaxation and reduced blood pressure via upregulation of the eNOS/sGC pathway

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Hydrogen sulfide (H2S), a gasotransmitter with protective effects in the cardiovascular system is endogenously generated by three main enzymatic pathways: cystathionine gamma lyase (CTH), cystathionine beta synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (MPST) enzymes. CTH and MPST are the predominant sources of H2S in the heart and blood vessels, exhibiting distinct effects in the cardiovascular system. To better understand the impact of H2S in cardiovascular homeostasis, we generated double Cth/Mpst knockout (Cth/Mpst-/) mouse and characterized their phenotype. CTH/MPST-deficient mice were viable, fertile and exhibited no gross abnormalities. Lack of both CTH and MPST did not affect the levels of CBS and H2S-degrading enzymes in the heart and the aorta. In line with the reduced expression of CTH and MPST, reduced protein persulfidation was evident in double knockout mice. Cth/Mpst-/- also exhibited reduced systolic, diastolic and mean arterial blood pressure, and presented normal left ventricular structure and fraction. Aortic ring relaxation in response to exogenously applied H2S was similar between the two genotypes. Interestingly, an enhanced endothelium-dependent relaxation to acetylcholine was observed in mice in which both enzymes were deleted. This paradoxical change was associated with upregulated levels of endothelial nitric oxide synthase (eNOS) and soluble guanylate cyclase (sGC) α1/β1 subunits and increased NO-donor-induced vasorelaxation. Administration of an eNOS-inhibitor increased mean arterial blood pressure to a similar extend in wild-type and Cth/Mpst-/- mice. We conclude that chronic elimination of the two major H2S sources in the cardiovascular system, leads to an adaptive upregulation of eNOS/sGC signaling, revealing novel ways through which H2S affects the NO/cGMP pathway.

Implementing pharmacogenetic testing in fluoropyrimidine-treated cancer patients: DPYD genotyping to guide chemotherapy dosing in Greece

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Aim: Fluoropyrimidines are widely used for the treatment of solid tumors. Approximately 10-30% of fluoropyrimidine-treated patients develop early-onset severe or life-threatening toxicity. Dihydropyrimidine dehydrogenase (DPD), encoded by DPYD gene, is the rate-limiting enzyme responsible for fluoropyrimidine catabolism. DPYD gene variants seriously affect DPD activity and are well validated predictors of fluoropyrimidine-associated toxicity. DPYD variants rs3918290, rs55886062, rs67376798 and rs75017182 are currently included in genetic-based dosing recommendations for fluoropyrimidines developed by the Clinical Pharmacogenetics Implementation Consortium. On March 2020, European Medicines Agency has recommended that patients receiving fluoropyrimidine therapy should be tested at least for these four DPYD variants before treatment initiation. In Greece, however, no data exist on DPYD genotyping. The aim of the present study was to analyze prevalence of DPYD rs3918290, rs55886062, rs67376798 and rs75017182 variants and assess their association with fluoropyrimidine-induced toxicity in Greek cancer patients.

Patients and methods: Study group consisted of 313 fluoropyrimidine-treated cancer patients. DPYD genotyping was conducted on QuantStudio ™ 12K Flex Real-Time PCR System (ThermoFisher Scientific) using the TaqMan® allelic discrimination assays C__30633851_20 (rs3918290), C__11985548_10 (rs55886062), C__27530948_10 (rs67376798) and C_104846637_10 (rs75017182).

Results: Any grade toxicity (1-4) was recorded in 208 patients (66.5%). Out of them, 25 patients (12%) experienced grade 3-4 toxicity. DPYD variants were detected in 9 patients (2.9%), all experiencing toxicity. This frequency was found increased in grade 3-4 toxicity cases (15.4%, p=0.005). DPYD deficiency increased risk for grade 3-4 toxicity (OR: 6.364, p=0.013) and for grade 1-4 gastrointestinal (OR: 15.213, p=0.011), neurological (OR: 4.462, p=0.029) and nutrition/metabolism (OR: 5.078, p=0.027) toxicities. Fluoropyrimidine dose intensity was significantly reduced in DPYD deficient patients (β=-0,093, p=0.010).

Conclusions: Our findings confirm the clinical validity of DPYD variations as risk factors for development of fluoropyrimidine-associated toxicities in the Greek population. Pre-treatment DPYD genotyping should be implemented in clinical practice and guide fluoropyrimidine dosing. Identifying additional DPYD polymorphisms can increase the prognostic value of DPYD genotyping and improve safety of fluoropyrimidine-based chemotherapy.

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Low receptor protein tyrosine phosphatase zeta 1 expression induces lung carcinogenesis and angiogenesis in vivo and predicts responsiveness to tyrosine kinase inhibitors

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Aim: In lung cancer patients, decreased expression of receptor protein tyrosine phosphatase zeta 1 (PTPRZ1) associates with poor prognosis and PTPRZ1 expression inversely correlates with overall patient survival. PTPRZ1 has been also shown to mediate the stimulatory effect of vascular endothelial growth factor A (VEGFA) and pleiotrophin (PTN) on endothelial cell migration, suggesting that it may also affect angiogenesis. The aim of the present study is to address the role of PTPRZ1 in lung carcinogenesis and angiogenesis in vivo.

Methods: In Ptprz1-/- and Ptprz1+/+ mice, we applied the in vivo model of urethane-induced lung adenocarcinoma development to study lung carcinogenesis and angiogenesis. Lung microvascular endothelial cells (LMVECs) were isolated from Ptprz1-/- and Ptprz1+/+ mice and used to study angiogenic properties and response to pharmacological mediators in vitro.

Results: PTPRZ1 deletion significantly enhanced chemically induced lung adenocarcinoma development, concomitant with increased alveolar macrophage infiltration and angiogenesis, and decreased animal survival. In agreement, lung microvascular endothelial cells (LMVECs) from Ptprz1-/- mice have decreased expression of β3 integrin, increased c-Met activity and enhanced angiogenic properties compared to Ptprz1+/+ LMVEC. The increased angiogenesis in vitro and in vivo and the enhanced lung carcinogenesis are inhibited by the c-Met inhibitor crizotinib, which also inhibits VEGFA- and PTN-induced endothelial cell migration, as well as the stimulatory effect of a selective PTPRZ1 tyrosine phosphatase inhibitor, suggesting that PTPRZ1 tyrosine phosphatase activity regulates endothelial cell activation and angiogenesis through c-Met.

Conclusions: Altogether, these data support the significant role of PTPRZ1 in lung adenocarcinoma development and angiogenesis through regulation of c-Met activation.

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Exploring the epigenetic factor UHRF1 as a potential target for achieving therapeutically relevant DNA hypomethylation

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Aim: Ubiquitin-like protein containing PHD and RING finger domains 1 (UHRF1) is a multi-domain protein regarded as a highly important hub in epigenetic regulation. This results from its key role in integrating signals deriving from DNA methylation and histone modification. The aim of the study is to identify small molecule inhibitors of UHRF1 and utilize them to provide evidence that the resulting hypomethylation and recovery of gene expression may have a therapeutic potential in pathologies where UHRF1 overexpression is prominent, such as in lung and bladder cancer and hepatocellular carcinoma.

Materials and methods: Compounds with structural similarity to 5-methylcytidine were computationally identified and ordered by the NCI repository. Rationally designed analogues were derived by organic synthesis. Evaluation of their in vivo DNA hypomethylation activity was based on experiments in D.rerio embryos treated with control and inhibitors at a range of concentrations around 80% from epiboly, DNA collection and global methylation quantification at 24 and 52 hours post fertilization.

Results: Previous results from our research group have identified the hypomethylation capacity of a uracil analogue discovered by in silico screening against the SRA domain of UHRF1. Further exploration of the previous hit was performed by a combined subscaffold search in the NCI repository, molecular simulations and organic synthesis. A number of analogues were developed and the most promising were subsequently evaluated for their DNA hypomethylating potential in vivo. Compounds 105 (NSC232005) and 106 resulted in a significant and dose dependent decrease of global DNA methylation. Specific phenotypic defects in the embryos were observed in high concentrations of 105 and it is anticipated that the inhibitors function by modulating UHRF1 activities related to development in both physiological or malignant context.

Conclusion: Utilization of a combined theoretical and experimental screening platform resulted in the identification of DNA hypomethylation molecules, affording proof of concept for the development of highly specific UHRF1 inhibitors as chemical probes and drug candidates. Chemical inhibition of UHRF1 is expected to enable the validation of this epigenetic factor as a drug target and it will facilitate mechanistic determination of its multiple features as a pivotal epigenetic regulator.

ΑΝΑΡΤΗΜΕΝΕΣ ΑΝΑΚΟΙΝΩΣΕΙΣ / POSTER PRESENTATIONS

The transcription factor COUP-TFII (NR2F2) controls Apolipoprotein AI and Apolipoprotein B expression in the HepG2 hepatocyte cell line

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Objective: Chicken Ovalbumin Upstream Promoter Transcription Factor II (COUP-TFII/NR2F2) is an orphan nuclear receptor that plays an important role in embryogenesis and adult life, regulating cell differentiation and fibrometabolic processes. Previous studies have suggested that COUP-TFII may act as a modulator of Apolipoprotein (Apo) AI and ApoB expression, based on its binding on their promoter regulatory elements, hence its original name, Apo-AI regulatory protein (ARP-1). However, direct data supporting this are entirely lacking. Our aim was to test in vitro how Apo-AI and ApoB levels are affected following COUP-TF II activity modulation.
Methods: In HepG2 hepatocytes, which express endogenously COUP-TFII as well as Apo-AI/ApoB, we either knocked-down COUP-TFII with 50nM of a specific siRNA or treated cells with 10μM of a reportedly selective small molecule inhibitor of COUP-TFII, synthesized and chemically characterized in place. After 24 or 48 hours, total RNA was used for the generation of cDNA libraries, followed by determination of Apo-AI and ApoB mRNA levels via RT-qPCR. Cell lysates and media supernatants were also analyzed via Western blotting for Apo-AI protein content.
Results: siRNA-mediated COUP-TF II knockdown (by ~40%) significantly increased both cell-associated and cell medium Apo-AI protein levels at 48h after transfection, by 47% and 49%, accordingly. mRNA levels of Apo-AI were not significantly affected, while those of ApoB were reduced by 23%. In contrast, inhibition of COUP-TFII by CIA2 caused a statistically significant decrease in Apo-AI protein levels in both the cell lysate (-91%) and the supernatant (-80%). However, CIA-2, similarly to the siRNA, significantly downregulated ApoB mRNA levels by 36%, while Apo-AI mRNA levels were not significantly reduced (p=0.051).
Conclusions: Reduction of COUP-TFII levels (by siRNA) or its inhibition (by CIA-2) both result in modulation of Apo-AI and ApoB mRNA and protein. Discrepancies between siRNA and the inhibitor may be explained either by incomplete reduction of COUP-TFII mRNA by the si, or by potential non-specific effects of CIA-2. CIA-2 constitutes a useful pharmacological tool to achieve reduction in Apo-AI or ApoB in vitro and probe the role of COUP-TFII in atherometabolic processes. Its effects in vivo are also being investigated.

The effects of gabapentin and fluoxetine in an animal model of neuropathic pain

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Aim: Pain is a complex sensation, consisting of a sensory/discriminative component, as well as affective/motivational and cognitive aspects. The combined activity of several brain areas (including limbic and cognitive structures) is involved in the perception of pain. Chronic pain is long lasting pain that persist for over six months that is difficult to treat pharmacologically. Current accumulating evidence suggests that chronic pain is the result of plastic changes in the neural circuits of the brain areas involved in pain perception. Thus, the ensuing altered processing might lead to sensory hypersensitivity and allodynia (perception of pain to non-painful stimuli), hallmarks of chronic pain. In addition, the same alterations might also lead to comorbidities such as anxiety and depression. On the other hand, chronic anxiety and depression can exacerbate pain perception, therefore initiating a positive reinforcement cycle. In order to better understand the association of the sensory and the affective aspects of pain within such a cycle, we test anxiolytic and analgesic drugs on behavioral outcomes of pain in a mouse model of neuropathic pain.

Materials and Methods: The spared nerve injury (SNI) model was used in 3-month-old male mice. Mechanical hypersensitivity was tested with a set of von Frey filaments of different forces. Horizontal motility was assessed in an open field (OF) arena. Control animals received 0,9% NaCl i.p., Fluoxetine was injected i.p. at doses 10 mg/kg and 30 mg/kg, while Gabapentin at doses of 10 mg/kg, 20 mg/kg, 30 mg/kg and 100 mg/kg.

Results: Fluoxetine showed a dose dependent decrease in mobility in the OF test. In addition, 30 mg/kg injections resulted in an increase of mechanical sensitivity thresholds. Lower concentrations of Gabapentin did not affect mechanical thresholds, while 100mg/kg increased mechanical pain thresholds, without affecting much the OF activity.

Conclusions: Our results show that the sensory and affective components of pain can be partly dissociated pharmacologically during chronic pain. This also indicates that these drugs involve different sites of action and mechanisms, which points to a possible synergistic effect if combined.

Pimozide strengthens the anticancer effect of paclitaxel in in vitro and in vivo models of NSCLC

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Background: Lung cancer is the second most frequent cancer in people and smoking is the major risk factor, which accounts for 75-80% of lung cancer-related deaths. Several epidemiological studies reported significantly lower cancer incidence in male schizophrenic patients following antipsychotic treatment compared to general population, although these patients are usually heavy smokers and adopt dietary habits that are largely related to carcinogenicity. Currently, the therapeutic strategies that are followed in lung cancer are based on multidrug schemes.

Aim: The present study investigated the potential anticancer effect of pimozide and its role in the anticancer effect of a standard drug used in the treatment of lung cancer, paclitaxel (PTX).

Materials and Methods: For this purpose, NSCLC cells were treated with PIM alone or in combination with PTX. H460 cell proliferation was assessed using the SRB test, and the apoptosis induction was assessed following the Annexin V-PI protocol. The impact of the combined therapy was also assessed using an in vivo murine model, the NSCLC xenograft model-NOD/SCID. The impact of the combined treatment on cancer-related signaling pathways was assessed with Western Blot.

Results: The in vitro study indicated that PIM markedly strengthened the PTX-mediated reduction of H460 cell population. PIM also enhanced the PXT-mediated repression of tumor weight and size. Notably, sub-therapeutic doses of PIM reduced the IC50 of PTX. At signal transduction pathway level, it appears that the anticancer effect of the combined treatment is mediated by activation of the PI3K/Akt/GSK3 pathway.

Conclusion: The present data suggest that PIM potentially displays anticancer properties by inducing apoptotic mechanisms via activation of the PI3K/Akt/GSK3 pathway. Both, PIM and PXT, when administered in combination even in sub-therapeutic doses, show a strong anticancer effect in experimental NSCLC models. This finding, if confirmed in the framework of clinical studies, could ensure an improved anticancer outcome in the treatment of NSCLC with restricted side effects.

Oleuropein promotes neural plasticity and neuroprotection via PPARα activation

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Oleuropein (OLE), a main constituent of olive, displays a pleotropic beneficial dynamic in health and disease, based mainly on its antioxidant and hypolipidemic properties. OLE activates peroxisome proliferator-activated receptor (PPARα) in neurons and astrocytes, providing neuroprotection against noxious biological reactions. Current study investigated the effect of OLE in the regulation of neural plasticity indices, emphasizing on the role of PPARα. For this purpose, SV129 wild type (WT) and PPARα null mice were treated with OLE for three weeks. Furthermore, in the framework of an in vitro study, the potential neuroprotective and antioxidant effects of various PPARα agonists, such as fenofibrate (FEN), WY-14643 and OLE, were tested in SH-SY5Y cells differentiated to cholinergic neurons. These neurons were treated with the supernatant of CHO-7PA2 transfected cells containing β-amyloid proteins, alone or in combination with PPARα agonist. To investigate the potential antioxidant effect of PPARα agonists, the cholinergic neurons were treated with hydrogen peroxide along with the PPARα agonists for 48 hours. The cell viability and neural plasticity pathways were assessed using the MTS assay and Western Blot, respectively. Current findings revealed that chronic treatment with OLE up-regulated the brain-derived neurotrophic factor (BDNF) and its receptor TrkB in the prefrontal cortex (PFC) of mice via activation of ERK1/2, PKA/CREB and AKT signaling pathways. No similar effects were observed in the hippocampus. OLE did not affect the neurotrophic factors NT-3 and NT-4/5 in both brain tissues. Interestingly, FEN, a selective PPARα agonist, up-regulated BDNF and NT-3 in the PFC, whereas the drug induced NT-4/5 in both brain sites tested. The OLE-induced effects on BDNF and TrkB are mediated by PPARα, because no similar alterations were observed in Pparα-null mice. Furthermore, OLE appeared to display a mild neuroprotective effect as it protected the neurons from the toxic effects of β-amyloid proteins. These in vitro effects of OLE are in line with those detected in the PFC of WT mice. In conclusion, OLE and similar drugs acting as PPARα agonists could improve synaptic function and dendritic outgrowth mainly in the PFC and at a lesser extend in the hippocampus, with potential beneficial effects on cognitive functions.

Blockade of CB1 or activation of CB2 cannabinoid receptors affects the early pathological events of diabetic retinopathy

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Aim: The basic goal of the present study was to examine the effects cannabinoids, namely, the CB1R antagonist, SR141716, the CB2R agonist, AM1710, or a dual treatment, combining these two agents, in preventing the development of neuroinflammation and neurodegeneration at the early stages of diabetic retinopathy (DR).

Materials and Methods: We employed a two week rat model of streptozotocin-induced diabetes, that resembles the early stages of DR. SR141716 and AM1710 were administered topically, via eyedrops, once daily for fourteen days at a concentration of 10mg/ml, starting two days after induction of diabetes.The neuroprotective properties of the cannabinoid treatment were evaluated by immunohistochemical studies, using antibodies against retinal neuronal markers like brain nitric oxide synthetase (bNOS), nerve fiber layer (NFL) and cleaved caspase 3. Antibodies against nitrotyrosine, Iba1(microglia marker) and GFAP (maroglia marker) were employed to assess the cannabinoids' induced antioxidant and anti-inflammatory properties, respectively. The levels of the pro-inflammatory cytokine TNFa in the retina were also analyzed with an ELISA assay.

Results: Single cannabinoid treatment with the CB2R agonist, AM1710, restored the diabetes induced reductions in bNOS and NFL immunoreactivity, reduced the number of cleaved caspase 3 positive cells and preserved the thickness of the inner nuclear layer to normal levels. SR141716, was only effective in restoring NFL immunoreactivity and reducing nitrotyrosine expression in the diabetic retinas. AM1710 also displayed a strong anti-inflammatory profile, since it reduced activation of microglia and macroglia, as well as the levels of TNFa in the retina, while we did not observe the same effects by SR141716. The combination of AM1710 and SR141716 displayed some protective properties in bNOS and NFL immunoreactivity, but it was not more effective than the single treatments. However, the dual treatment displayed a higher efficacy in reducing nitrotyrosine expression.

Conclusions: Collectively, we provide new evidence regarding the neuroprotective actions of cannabinoids, when administered topically via eyedrops, against the diabetes - induced oxidative damage, neurodegeneration and neuroinflammation observed in the early events of diabetic retinopathy.

Effect of the novel NOX2 inhibitor, GLX7013170, in experimental animal models of retinopathies

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Aim: The present study aimed to investigate the involvement of NOX2 isoform of NADPH oxidase in retinal pathologies, associated with excitotoxicity and diabetes, through the actions of the novel NOX2 inhibitor, GLX7013170.
Methods: Sprague-Dawley rats were used for the induction of two in vivo retinal models: AMPA excitotoxicity and streptozotocin (STZ) induced Diabetic Retinopathy (DR). The in vivo AMPA excitotoxicity model was established through intravitreal administration of AMPA (42nmoles/eye, 8.4mM), AMPA+GLX7013170 (NOX2 inhibitor,10-4M), or vehicle (control group). For the in vivo model of DR, rats were intraperitoneally administered with STZ (70mg/kg), 48 hours later a 14-day treatment began: rats received vehicle or GLX7013170 (10mg/ml, dissolved in DMSO), as eye drops (20µl/eye), once daily. Immunoreactivity studies using antibodies raised against: a) neuronal retinal markers nitric oxide synthase (bNOS, amacrine cells, AMPA model) and neurofilament (NFL, Retinal Ganglion Cells axons, DR model) and b) inflammatory markers glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule-1 (Iba-1), were employed, to assess the effect of NOX2 inhibition on retinal neurons and on macro/microglia in both in vivo models.
Results: In the in vivo model of AMPA excitotoxicity, GLX7013170 was able to protect the bNOS positive amacrine cells against AMPA induced toxicity and also attenuated the activation of macro- and microglia, as shown by the reduction in GFAP and Iba-1 immunoreactivities, respectively. In the STZ model of DR, the NOX2 inhibitor did not exhibit any protection against the diabetes induced insult on the axons of retinal ganglion cells, but it was able to reduce the over expression of GFAP and limit the number of activated Iba-1 positive cells in the retinas of diabetic treated animals, compared to the non-treated ones.
Conclusions: The aforementioned results suggest that the NOX2 isoform is implicated in the development of retinal pathologies linked with excitotoxic insults and diabetes and that the NOX2 inhibitor, GLX7013170, provides neuroprotection to the retina, due to its anti-inflammatory and neuroprotective (bNOS amacrine cells) actions.

Topically administered NOX4 inhibitor, GLX7013114, protects the rat retina against the early pathological events of diabetic retinopathy

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Aim: The aim of the present study was to investigate the role of the novel NOX4 inhibitor, GLX7013114, in the early events of diabetic retinopathy (DR) and thus, to evaluate its efficacy as a therapeutic agent for the treatment of DR.

Methods: Sprague-Dawley rats were used in two in vivo experimental streptozotocin (STZ) induced DR paradigms (A,B), both depicting the early events of the disease. A single dose of STZ (70mg/kg) was intraperitoneally administered to rats for the induction of diabetes. In paradigm A, 48 hours after STZ administration a 14-day treatment began, while in paradigm B, a 7-day treatment started 4 weeks post STZ injections. In both paradigms, rats received once daily vehicle or GLX7013114 (10mg/ml, dissolved in DMSO), as eye drops (20μl/eye). Immunohistochemical studies were performed, using specific markers against nitrotyrosine, caspase-3, neurofilament (NFL, Retinal Ganglion Cells-RGC axons), nitric oxide synthase (bNOS,amacrine cells), ionized calcium-binding adaptor molecule-1(Iba-1, microglia) and glial fibrillary acidic protein (GFAP, macroglia). Western blot analysis was employed for the determination of vascular endothelial growth factor (VEGF) levels in retinal samples, while Real time PCR and ELISA analyses were used for the quantification of NOX4 isoform, IL-1β, IL-6 mRNA levels and TNF-α protein levels, respectively. PERG analysis was performed to further evaluate the function of RGCs in paradigm B.

Results: Diabetes caused a significant increase in the expression levels of the NOX4 isoform in rat retina. GLX7013114, topically administered as eye-drops managed to reduce oxidative nitrative stress and activation of caspase-3, micro/macroglia in both paradigms of DR. It also protected RGCs axons and bNOS positive amacrine cells and attenuated the diabetes induced increase in the levels of VEGF and proinflammatory cytokines TNF-α (protein), IL-1β and IL-6 (mRNA). PERG analysis in Paradigm B supported that GLX7013114 (10μl/eye) also protected RGC function.

Conclusions: This study suggests that NOX4 isoform is implicated in the pathophysiology of the early stages of DR and the novel NOX4 inhibitor, GLX7013114, topically administrated as eye-drops, has the pharmacological profile of a promising therapeutic for the disease.

The nuclear receptor COUP-TFII (NR2F2) controls inflammatory responses in vascular endothelial cells in vitro

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Objective: The orphan nuclear receptor Chicken Ovalbumin Upstream Promoter Transcription Factor II (COUP-TFII/NR2F2) is endogenously expressed in the venous (but not arterial) endothelium and modulation of its expression correlates with severity of inflammatory responses. For this reason, our aim was to characterize how COUP-TFII modulates the response of vascular endothelial cells to proinflammatory stimuli in vitro.
Methods: We transfected primary Human Umbilical Vein Endothelial Cells (HUVECs) with 25nM of Control or specific COUP-TFII siRNAs. Subsequently, we treated the cells with TNF-α or IL-1β (0.1-10ng/mL) and determined: a) p65 (NFκB) protein phosphorylation (activation) via Western Blotting (20min post-treatment), b) surface expression of ICAM-1 and P/E Selectin leucocyte adhesion molecules by ELISA (4hrs post-treatment), and c) adhesion of calcein-loaded U937 leukocytes to HUVEC monolayers by fluorescence emission (4hrs post-treatment). In addition, we monitored COUP-TFII protein levels following exposure of HUVECs to TNF-α or IL-1β for 24hrs.
Results: Transfection with the specific siRNA reduced COUP-TFII protein levels by >90% for at least 72hrs. COUP-TFII silencing significantly: a) upregulated by 3-fold the phosphorylation of p65 in response to both TNF-α or IL-1β, b) further raised by 2- to 7-fold the induction of HUVEC surface ICAM-1 and P/E Selectin by the two cytokines, and c) increased by 3- to 6-fold the cytokine-induced adhesion of U937 to HUVECs. Finally, exposure to TNF-α and IL-1β diminished endothelial COUP-TFII protein levels by >50%.
Conclusions: We conclude that endogenous COUP-TFII expression in venous endothelial cells opposes multiple responses to the important proinflammatory cytokines TNF-α and IL-1β. Simultaneously, long-term endothelial exposure to TNF-α and IL-1β efficiently reduces COUP-TFII levels, further contributing to the exacerbation of endothelial inflammatory responses. Manipulation of COUP-TFII levels and/or activity could therefore be a means of therapeutic interference in disorders characterized by vascular inflammation.

Pharmacological properties of novel GnRH analogues conjugated with anthraquinone

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Aim: The receptors (GnRH-R) for the gonadotropin releasing hormone GnRH, in addition to their essential role in the function of the reproductive system are also highly expressed in different types of cancer cells. Previous studies have shown that GnRH analogues exert antiproliferative actions in various cancer cells, through their interaction with the GnRH-R expressed in these cells. In the present study we aimed to design, synthesize and pharmacologically characterize novel GnRH analogues conjugated with anthraquinone (con1-con8). These analogues are anticipated to have cytotoxic properties by releasing into cancer cells the anthraquinone group after their interaction with the GnRH-R expressed in these cells and their subsequent internalization in complex with the receptor.

Materials and Methods: We created the con1-con8 analogues by modifying the GnRH analogue, leuprolide and conjugated it with anthraquinone or its derivative, mitoxantrone. To evaluate the pharmacological properties of GnRH analogues we determined their binding affinities in competition radioligand binding studies using membrane homogenates from HEK 293 cells stably expressing the GnRH-R, and the [125I]-DTyr6-His5-GnRH as radioligand. Data were analyzed by nonlinear regression analysis and apparent binding affinities (IC50 values) were obtained by fitting the data to a one-site competition model.

Results: All compounds decreased the specific binding of 125I]-DTyr6-His5-GnRH in a dose-response manner, with affinities (0.04-3.5 nM) higher or similar to that of leuprolide (control, 0.6 nM). The compounds with the highest binding affinities were the con3, con6 and con7, which had affinities of 0.06 nM, 0.07 nM and 0.04 nM, respectively. In contrast to con1-con8, mitoxantrone did not bind to the GnRH-R.

Conclusions:

1) The GnRH analogues (con1-con8) conjugated with anthraquinone or its derivative, mitoxantrone, bind to GnRH-R with high affinities, similar to or higher than leuprolide. In contrast, mitoxantrone did not bind to the GnRH-R.

2) Con3, and con7, which bear the cytotoxic mitoxantrone had higher affinities than the other analogues. Additional studies will determine the antiproliferative effects of these analogues.

Antiproliferative effects of a novel GnRH analogue conjugated with mitoxantrone

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Aim: The gonadotropin releasing hormone GnRH is a decapeptide that plays a key role in the function of the reproductive system through its interaction with the GnRH receptor (GnRH-R). GnRH-R is also highly expressed in different types of cancer cells, including the ovarian ones. Previous studies have shown that GnRH analogues exert antiproliferative actions in ovarian and other cancer cells, through their interaction with the GnRH-R expressed in these cells. In this study we aimed to develop novel GnRH analogues with enhanced antiproliferative actions, by conjugating them with the cytotoxic agent, mitoxantrone, thus creating the analogue Con7. Con7 is anticipated to release mitoxantrone into cancer cells after its binding to GnRH-R and subsequent internalization of the GnRH-R/Con7 complex.

Materials and Methods: To create the con7 analogue we chemically modified the GnRH analogue, leuprolide and conjugated it with mitoxantrone. To evaluate the antiproliferative properties of con7 we incubated the ovarian cancer cells, SK-OV-3, with con7 at different concentrations for 1-4 days and used the MTT assay.

Results: Proliferation of SC-OV-3 cells was inhibited by Con7 in a time-dependent manner (1-4 days). At longer exposure of cells to con7 the concentration of peptide required to decrease cell viability was smaller than that required to decrease viability to a similar degree at shorter exposure (n=4). For example, the decrease of cell viability by 20% after their exposure to 10 nM of con7 for 4 days, was similar to that (27%) after exposure of cells to 1000 nM of con7 for 1 day. Importantly, proliferation of SC-OV-3 cells was also inhibited by Con7 in a dose-dependent manner. The antiproliferative potency of con7 after exposure of SC-OV-3 to peptide for 1,2,3 and 4 days, was 1.4 μM, 0.72 μM, 0.97 μM and 0,83 μM (n=4), respectively.

Conclusions:

1. Proliferation of SC-OV-3 cells was inhibited by Con7 in a time-dependent manner reaching maximum inhibitory effect at day 4.
2. Proliferation of SC-OV-3 cells was inhibited by Con7 in a dose-dependent manner at all days examined.
3. The antiproliferative potency Con7 increased from 1.4 μM at day 1 to 0.83 μM at day 4

A novel GnRH analogue conjugated with mitoxantrone as an antiproliferative agent in breast cancer cells

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Aim: The gonadotropin releasing hormone GnRH is a decapeptide known for its pivotal role in the physiology of the reproductive system through its interaction with the GnRH receptor (GnRH-R). Importantly, GnRH-R is highly expressed in various types of cancer cells, such as breast cancer cells. Previous studies have shown that GnRH analogues exert antiproliferative actions in breast cancer cells, through their interaction with the GnRH-R. In this study we aimed to develop novel GnRH analogues with enhanced antiproliferative actions, by conjugating them with the cytotoxic agent, mitoxantrone, thus creating the analogue Con3. Con3 is anticipated to release mitoxantrone into cancer cells after its binding to GnRH-R and subsequent internalization of the GnRH-R/Con3 complex.

Materials-Methods: To create the con3 analogue we chemically modified the GnRH analogue, leuprolide and conjugated it with mitoxantrone. To evaluate the antiproliferative properties of con3 we incubated the breast cancer cell line MDA-MB-231, with con3 at different concentrations for 1-4 days and the proliferation rate was measured by the MTT assay. In addition, we investigated the effect of con3 in cell motility/migration in a concentration (〖10〗 ^(-6)M) for 6, 12 and 24hours using the scratch assay.

Results: Proliferation of MDA-MB-231 cells was inhibited by Con3 in a time-dependent manner (1-4 days). The antiproliferative effect of con3 was statistically significant at day 3 and 4. In addition, con3 did not increase cell motility/migration in MDA-MB-231 cancer cells at any time point.

Conclusions: Proliferation of MDA-MB-231 cells was inhibited by Con3 in a time-dependent manner, reaching maximum inhibitory effect at day 4. Moreover, Con3 did not accelerate cell motility/migration, indicating a potential cytostatic role in breast cancer cells.

Elucidating the behavioral and mitochondrial correlates of early handling

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Aim: Early life manipulations are studied in rodents to disentangle their persistent effects in adulthood. Early Handling (EH) is an early life intervention consisting of the brief and repeated separation of the pups from their mother during the first days after birth. Our study aimed to explore the effects of EH on maternal behavior as well as on male and female pup behavior in adulthood and to investigate the implication of brain mitochondria in modulating the effects of EH in the offspring.

Material-Methods: To address these questions in different anxiety backgrounds, we used high anxiety-related behavior (HAB) and normal anxiety-related behavior (NAB) mice. We investigated the effects of EH in maternal behavior in HAB and NAB mice by observing dams from postnatal day 2 to 7. We studied the EH effects on HAB and NAB pup behavior by performing a behavioral test battery (social preference-avoidance test, dark-light box test, open field test, forced-swim test). For our molecular analysis, we used Western blots and biochemical assays to detect changes in mitochondrial housekeeping and metabolic functions and real-time qPCR to examine mRNA level alterations in mitochondrial dynamics, a term collectively addressing the molecular machinery of mitochondrial biogenesis, fission, fusion and mitophagy.

Results: EH did not affect maternal behavior within the HAB and NAB lines, however, we found significant differences between the basal (non-handling group, NH) HAB and NAB dams. Interestingly, EH exerted anxiolytic effects in dark-light box test in HAB-EH compared to HAB-NH male pups. Following up these results at the molecular level in the prefrontal cortex and hippocampus of HAB male pups, we found that EH resulted in mRNA level alterations of key players of the mitochondrial dynamics machinery in HAB-EH compared to HAB-NH male pups. Protein levels of mitochondrial housekeeping functions were not affected by EH in HAB male pups.

Conclusions: Overall, these findings highlight an implication of mitochondrial dynamics in the anxiolytic effects of EH in HAB male mice. Unraveling the behavioral and molecular mechanisms implicated in EH effects and elucidating how EH influences adult behavior may facilitate the discovery of candidate biomarkers and therapeutic targets for anxiety disorders.

Oncostatin M, a novel therapeutic target for intestinal inflammation and fibrosis, induces pro-inflammatory- and fibrotic-related marker expression on human intestinal organoids

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Aim: Our aim was to investigate Oncostatin M (OSM) as a possible therapeutic target for intestinal inflammation and fibrosis, through the examination of its effect on the expression of pro-inflammatory and fibrotic markers on Human Intestinal Organoids (HIOs), with and without the combined presence of IL-1α and TNF-α.

Materials & Methods: HIOs were developed from the human embryonic stem cell line (H1) using a commercially available kit and were later characterized by immunofluorescence. HIOs from passage 2 were stimulated with either 100ng/ml OSM for 12 hours or 5ng/ml IL-1α and 50ng/ml TNF-α for 24 hours and then with 100ng/ml OSM for 12 hours. mRNA expression of fibrotic markers, Collagen Type I, III, and Fibronectin, and pro-inflammatory markers, CCL2, CXCL10 and CXCL11 were examined by reverse transcription quantitative PCR.

Results: OSM alone significantly downregulated both Collagen Type I (0.28-fold, ±0.06, $p<0.01$) and III (0.31-fold, ±0.03, $p<0.0001$) mRNA expression, and had no effect on Fibronectin expression. When HIOs were previously exposed to IL-1α and TNF-α, however, OSM stimulation resulted in significant upregulation of Collagen Type I (1.77-fold, ±0.40, $p<0.05$) and Fibronectin (3.13-fold, ±0.29, $p<0.0001$), and had no effect on Collagen Type III. Regarding the pro-inflammatory marker expression, OSM alone induced the expression of CCL2 (1.25-fold, ±0.20), CXCL10 (1.69-fold, ±0.24, $p<0.01$) and CXCL11 (1.40-fold, ±0.12, $p<0.01$), with a greater effect observed when HIOs were previously exposed to IL-1α and TNF-α (CCL2: 3.57-fold, ±0.48, $p<0.0001$; CXCL10: 4.14-fold, ±0.71, $p<0.0001$; CXCL11: 2.33-fold, ±0.41, $p<0.001$).

Conclusions: Our findings indicate that OSM induces the expression of both pro-inflammatory and fibrotic factors in early passages of HIOs, previously exposed to IL-1α and TNF-α, and therefore, suggesting that OSM could be a promising therapeutic target for intestinal inflammation and fibrosis. In addition, since the mesenchymal cell component of HIOs is strongly active in early passages and the OSM's receptor is mainly found to be expressed in these cells, our results suggest that OSM affects HIOs via the cells that are of mesenchymal origin.

Inflammation induces the expression of TrkB and p75 neurotrophin receptors in human colonic subepithelial myofibroblasts

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Aim: Our aim was to investigate the expression of the neurotrophin receptors TrkA, TrkB and p75NTR in healthy human colonic subepithelial myofibroblasts (cSEMFs) stimulated with the pro-inflammatory cytokines IL-1α and TNF-α. To our knowledge, neurotrophin receptors have not been studied in this cell type, while their expression has been detected in many epithelial and immune cells.

Materials & Methods: Human cSEMFs were isolated from endoscopic biopsies, set to culture and later stimulated with 5ng/ml IL-1α and 50ng/ml TNF-α for 24 and 48 hours. At the end of the incubation periods, supernatants were collected and total cells were harvested in lysis buffer. The protein expression of the neurotrophin receptors TrkA, TrkB and p75NTR was detected in lysed cells and proper controls using Western blot analysis.

Results: Non-stimulated cells were lacking the expression of all neurotrophin receptors (TrkA, TrkB and p75NTR), while stimulation with IL-1α and TNF-α (2C) for 24 and 48h led to a significant and time-dependent increase in the protein expression of the TrkB (3-fold increase) and p75NTR (4-fold increase) compared to unstimulated cSEMFs.

Conclusions: Our results show that the expression of TrkB and p75NTR neurotrophin receptors is induced upon inflammatory challenges in cSEMFs, indicating a significant role on this process. Our in vitro approach could mimic the microenvironment of inflamed mucosal tissue of patients with Inflammatory Bowel Diseases or related disorders, and thus to be proven useful as an in vitro platform for an initial drug screening of novel or repurposed agents against these diseases. Conclusively, we propose a new pharmacological approach for evaluation of ligands of these neurotrophin receptors as potential anti-inflammatory compounds.

Expression of p75 neurotrophin receptor and its role in adult mouse and human neurogenesis: a novel therapeutic target against Alzheimer's Disease

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Aim: The pan-neurotrophin p75 receptor (p75NTR) is a member of the TNF death receptor superfamily, widely expressed in many cell types, including adult neural stem cells (aNSCs). Its remarkable up regulation during neurodegeneration and its controversial signaling, ranging from survival to cell death, have attracted a special interest on this receptor as a potential pharmacological target against Alzheimer's Disease (AD). Recent studies are also highlighting the importance of this receptor on the adult hippocampal neurogenesis, which drops sharply in AD and remains poorly understood. Furthermore, to date, there is no study addressing its role in human neural stem cells (hNSCs).

Materials and Methods: We focus on revealing the p75NTR pleiotropic functions, by examining adult hippocampal neurogenesis levels on p75NTR null and p75NTRflox/Nestin-Cre mice and identifying aNSCs proliferation and survival, using immunohistochemical methods. In addition, we use human induced Pluripotent Stem Cells (hiPSCs)-derived NSCs to investigate the role of p75NTR in human neurogenesis.

Results: p75KO mice exhibit decreased NSCs proliferation as indicated by the number of BrdU+/Sox2+ cells and attenuated neuronal differentiation in the hippocampal Dentate Gyrus (DG) suggesting key neurogenic properties of p75NTR. We have also generated NSCs from human iPSCs derived from healthy individuals and AD patients (ApoE4 mutation). We examined changes in expression level and activity of p75NTR, showing active receptor's signaling and regulation of survival in the presence of neurotoxic Amyloid-β peptides.

Conclusion: In summary, our results from p75KO mice suggest receptor's requirement for intact neurogenesis. The present study also demonstrates for the first time the expression and activity of p75NTR in human NSCs and indicates p75NTR involvement in AD pathology. Deciphering the specific signaling pathways necessary to mediate the actions of p75NTR on aNSCs' properties, we aim to reinforce endogenous ability of neurogenesis and thus strengthening its repairing capacity against AD-induced neuronal loss.

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Glucocorticoid administration in astrocytes initiates a biphasic response on brain-derived neurotrophic factor expression.

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Aim: Both glucocorticoids (GCs) and neurotrophins, like brain-derived neurotrophic factor (BDNF), are strongly implicated in the pathophysiology of stress-related diseases through their defined effects on adult hippocampal neurogenesis and synaptic plasticity. Functional interactions between BDNF and GCs have been recently demonstrated indicating a potential synergy or antagonism on neuroplasticity under physiological or neuropathological conditions.

Materials and Methods: In order to further investigate this neuro-immunological crosstalk, we examined the baseline effects of a physiologically-relevant GC concentration on BDNF expression -both in mRNA and protein level- in primary cultures of astrocytes, in consecutive timepoints during a 24-hour exposure.

Results: Our results characterize in astrocytes the existence of two temporally distinct functions with opposing outcomes on BDNF expression: an early, very fast effect that occurs within the first hour of GC administration and leads to BDNF overexpression and a late inhibitory effect, that can last for up to 24 hours. Using selective pharmacological inhibitors, we were able to match the involvement of Glucocorticoid receptor to the late effect and interestingly, the necessity of both Mineralocorticoid receptor and TrkB (BDNF receptor) for the regulation of the early effect. Finally, by simulating in vitro the physiological pulsatile secretion of GCs, we demonstrated astrocyte's ability to restore BDNF expression, back to its baseline untreated level, thus highlighting the well-established homeostatic capabilities of GCs and the important role of astrocyte susceptibility to GC pulsatility.

Conclusion: Our results depict the differential effects of glucocorticoid rhythmicity on the BDNF neurotrophin expression, indicating a complex interaction between the two major regulatory systems of inflammation and neuronal function, and thus introducing new avenues for pharmacological interventions in many neurological disorders.

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Cracking down on addiction: The biotechnological approach

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Aim: Current pharmacological substance use disorder (SUD) treatments favor a pharmacodynamics approach, employing agonists/antagonists of receptors. Their limitations include reliance on long term patient compliance, on target off site effects, unavailability for many drugs of abuse (DOA) and sometimes perpetuation of the addiction. A pharmacokinetic approach barring blood-brain barrier penetration of the DOA or a treatment which targets intracellular addiction mechanisms, constitute attractive alternative approaches. Our aim was to examine the literature on whether relevant biopharmaceutical and biotechnological approaches could deliver a fine-tuned solution with less side effects compared to treatments currently available.

Methods: A narrative review was compiled, spanning the last 20 years. We scanned major databases such as Scopus and PubMed for appropriate articles. Articles concerning traditional approaches such as drug substitution or receptor antagonism were excluded, whereas included items had a biopharmaceutical or biotechnological component.

Results: Our search yielded approaches recruiting passive and active immunization against DOA, metabolic enhancers augmenting drug metabolism/clearance, and genetic/epigenetic modulations of key genomic elements implicated in addiction. Active immunization relies on production of antidrug antibodies by means of patient vaccination, using a hapten conjugated DOA, while passive immunization constitutes of exogenous administration of such antibodies. Metabolic enhancers include drug-specific metabolizing enzymes, as well as catalytic antibodies that increase metabolic liability of DOAs. Lastly, nanotechnology can be used to genetically engineer or epigenetically modify “cornerstone” targets, common in all addictions, attenuating drug seeking behavior and reversing drug-induced brain changes.

Conclusions: of the above, only nicotine and cocaine vaccines have entered human trials, failing to promote abstinence. Improved hapten design, adjuvant selection and vaccination protocols are required to achieve optimal results. Metabolic approaches have yielded promising results in animal models. Lastly, intervention on genetic or epigenetic level, while invasive can be a promising solution to the polydrug abuse problem.

Olive-derived bioactive compounds salvage the myocardium from ischemia/reperfusion injury, via mechanisms involving apoptosis mediators and antioxidant enzymes.

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i.Aim: As accumulating clinical and preclinical data converge to the protective effects of olive derivatives in cardiovascular disease, we focus on four olive-derived bioactive compounds, oleuropein(OL), hydroxytyrosol(HT), oleocanthal(OC), and oleanolic acid(OA) aiming to investigate their possible cardioprotective effects against ischemia-reperfusion injury (IRI) in a mouse model of metabolic syndrome (MS).

ii.Materials and Methods: To establish the MS, C57Bl mice were fed with Western diet for 14 weeks. On week 8, animals were randomized into 6 groups: i) Normal Saline, ii) OL (20.6 mg/kg), iii) HT (5.9 mg/kg), iv) DMSO 5% v) OC (11.6 mg/kg), vi) OA (17.4 mg/kg), receiving the assigned compound/vehicle daily by oral gavage for the last 6 weeks of the experimental protocol. At baseline, on weeks 8 and 14, the body weight, fasting blood glucose and lipidemic profile were evaluated, as fundamental parameters of the metabolic-syndrome pathologies. At the 14th week, mice were subjected to IRI by LAD ligation for 30min followed by 2h reperfusion. Then, the heart was excised and either stained with Evans blue and TTC for infarct size measurement, or frozen for western blot analysis of cardioprotective signaling pathways.

iii.Results: OL, OC and OA reduced the infarct size, with OC exerting the most potent cardioprotective effect. The observed protection was confirmed by lower Bax/Bcl-XL expression ratio, revealing an antiapoptotic effect. Furthermore, OC potentiated the expression of antioxidant enzyme SOD. MS establishment was confirmed by elevated fasting glucose, body weight and total cholesterol levels in control group at 14 weeks. Treatment with OL significantly reduced fasting glucose as well as LDL cholesterol, while HT lowered plasma triglycerides. Finally, OA restored total cholesterol but none of the treatments affected the increased body weight.

iv.Conclusions: Chronic treatment with OL, OC or OA confers cardioprotection in mice with MS, which is attributed to hampering of the intrinsic apoptotic pathway and additive amplification of antioxidant mechanisms by OC. In parallel, OL ameliorates hyperglycemia and lowers LDL cholesterol, while HT reduces triglycerides and OA reverses hypercholesterolemia. Elucidation of the molecular mechanisms and possible additive effects in combined treatments could emerge novel cardioprotective approaches and further benefit patients with MS.

Dihydromyricetin: a natural flavonoid could prevent hepatotoxicity and nephrotoxicity induced by Methotrexate treatment.

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Aim: The present study aims to examine the potential hepatoprotective and nephroprotective effect of Dihydromyricetin (DHM) against Methotrexate (MTX)-induced toxicity and to investigate the implicated molecular mechanisms.

Materials and Methods: First, a suitable mouse strain for our toxicity study was determined. Male BALBc and 129/SV mice were treated with a single dose of MTX (20mg/kg) i.p. After 24h, plasma hepatotoxicity biomarkers (AST, ALT) were evaluated. 129/SV mice- which emerged as a preponderant model- were randomized into 6 groups: 1)Control, 2)MTX, 3)DHM 100mg/kg, 4)DHM 200mg/kg, 5)MTX+DHM 100mg/kg, 6)MTX+DHM 200mg/kg. DHM was administered orally for 5 days, whereas MTX i.p. on day 5. 24h later, blood was collected and serum biomarkers of hepatotoxicity (AST, ALT, ALP, LDH) and nephrotoxicity (Creatinine, urea) were measured. Liver and kidney tissues were harvested for molecular analysis and determination of oxidative stress markers (MDA, Protein Carbonyls).

Results: 129/SV mice were more susceptible to MTX hepatotoxicity. Treatment with DHM in both tested doses restored the increased plasma levels of AST and ALT, while ALP and LDH levels were not significantly altered by MTX. Also, the potential renal toxicity indicated by increased creatinine (but not urea) levels was ameliorated by DHM. Additionally, although MDA levels remained unaffected, DHM reduced the MTX-induced increase in protein carbonylation in liver and kidney tissues. Western blotting analysis revealed that MTX-induced hepatotoxicity is mediated mainly through inflammation, activation of JAK/STAT3 and NF-kB and apoptotic cell death. DHM reversed these effects and activated the anti-apoptotic Bcl-XL. Its protection was not associated with PI3K/AKT/eNOS or Nrf2-antioxidant pathways. Renal toxicity was mainly attributed to inflammation, as indicated by the increased expression of Il-6 and phosphorylation of NfkB and STAT-3. In our model, the apoptotic and oxidative pathways were involved to a less extent in MTX's nephrotoxicity. DHM reduced the levels of the aforementioned signaling molecules contributing to a nephroprotective effect.

Conclusions: In both organs, DHM attenuated the activation of critical pathways responsible for the deleterious effects of MTX. Consequently, DHM exerts hepatoprotective and possibly nephroprotective effects against MTX-induced toxicity and these beneficial properties could render DHM as a great candidate for supporting patients receiving MTX therapy.

A Comparison of EMA and FDA Decisions for New Drug Marketing Applications 2015–2021

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Aim: Over the last decades, the comparison between FDA and EMA regarding the approval of new drugs and their market release has been intense. Aim of the present study was to compare all marketing applications
for new active substance, a new chemical entity, or a therapeutic biologic product submitted to the FDA and the EMA which had an outcome in the regulatory period 2015- 2021.
Methods: Collecting the data from FDA and EMA public websites, the joint applications submitted to the EMA and the FDA were grouped and the timing of the applications, the therapeutic field, the application approval route and the initial and final outcome were studied. The percentage of concordance and discordance was determined and the reasons for discordance were grouped and assessed.
Results: The reasons for non-approval were common between the agencies including safety, efficacy and the quality of the product. The main reason for a rejection by the EMA was the lack of documentation regarding the efficacy of the drug. Secondary reasons were safety and the unsatisfactory presentation of data. No applications found that were rejected due to quality of the products. Based on our findings the most important reason for the FDA was the quality that in the majority of the applications concerned the manufacturing facilities. In terms of timeline, the differences were mainly observed due to the type of application submitted to each organization. Although both the FDA and the EMA have developed pathways that lead to the acceleration of the approval process, it appears that EMA follows the standard procedure in the majority of applications. On the contrary, FDA includes most of the applications in the corresponding categories of accelerated approval aiming to get the drugs on the market as quickly as possible. Finally, differences were noticed in terms of flexibility in evaluating the most important parameters of the application since the role of subjective judgment still governs the evaluation procedures.
Conclusions: A possible explanation of the differences found between the organizations is that EMA uses a mixed qualitative/ quantitative benefit-risk evaluation model while the FDA uses a more qualitative approach.

Emergency drugs that are used in the dental office

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Aim: Medical emergencies in the dental office are an unavoidable part of the profession and may range from relatively benign conditions to life-threatening situations. The purpose of the present study is to provide a literature review on the basic and critical drugs and equipment necessary for the staff members in general dental offices to manage the most common and anticipated medical emergencies.
Materials and Methods: A thorough literature search was performed in Pubmed (Medline) and Scopus (ELSEVIER) using the following terms in every possible combination: “medical”, “emergency”, “dental” and “office”. Inclusion criteria were (1) original reports, reviews and letters, (2) written in the English language and (3) published from 2012 to 2022. The two authors conducted the literature search independently. The reviewers discussed any discrepancies about the inclusion or exclusion of studies until consensus was reached.
Results: The level of agreement between the two reviewers was 90.69% (Cohen’s kappa = 0.751; 95% CI 0.619, 0.883). Among the 812 articles found in Pubmed and Scopus, 26 studies were included in the review. The most common emergencies in Dental Offices are syncope 35.90%, anaphylactic shock/allergies 16.20%, hypoglycaemia 15.5%, cardiovascular problems 13.20%, asthma 8% and epileptic seizures 11.10%. The essential drugs used in these emergencies are: (1) oxygen, (2) epinephrine, (3) nitroglycerine, (4) injectable antihistamine (diphenhydramine), (5) bronchodilators (albuterol, salbutamol), (6) aspirin, (7) oral carbohydrates (as in orange juice), (8) aromatic ammonia. Other drugs considered as part of an emergency kit are glucagon, atropine, ephedrine, corticosteroids, morphine, naloxone and injectable benzodiazepines. In addition to drugs, basic equipment including a stethoscope, blood pressure cuff, syringes and needles should be readily available. Finally, it is essential before any kind of treatment to take a history of the patient, do a physical examination and check the vital signs. Basic life support (BLS) training for all staff is required.
Conclusions: A knowledgeable and skilled dental team together with a basic drug emergency kit and equipment constitute the essential conditions to make the dental office a safe environment for patients and ensure competent and timely aid in case of emergency.

Factors predicting PANSS score variability in patients under clozapine treatment: the role of CYP1A2 phenotyping

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Aim: Clozapine (atypical antipsychotic) is considered the drug of choice for the treatment of refractory schizophrenia. Fluctuations in serum levels of clozapine may alter the clinical status of patients. The main enzyme that metabolizes clozapine to norclozapine, of CYP1A2, is influenced by genetic and environmental (drugs, foods, smoking) factors. The aim of the present study was to examine a possible correlation between clozapine serum levels and CYP1A2 activity as a possible source of variability in the clinical response in patients with treatment refractory schizophrenia.

Materials and Methods: Twelve patients (age 42±11 yrs, 9 males, 5 smokers) diagnosed with schizophrenia with ICD 10 criteria, under treatment with clozapine (mean dose 296±121 mg/d, range 100-450) for at least 6 months, were included in the study. The severity of the disease was assessed by PANSS (Positive and Negative Syndrome Scale) (mean score 75±25, range 40-117). CYP1A2 activity was determined by caffeine metabolite ratios (CMRs) in urine ((AFMU+1U+1X)/17U) and saliva (17MU/137MX). Urine and saliva samples were collected 6 hours after ingestion of a 150 mg caffeine capsule concomitantly with a trough blood sample. The concentrations of caffeine metabolites in urine and saliva and those of clozapine and norclozapine in serum were determined by reversed-phase high-pressure liquid chromatography (RP-HPLC-UV) with detection at 280 and 215nm, respectively.

Results: High positive correlation was observed between norclozapine/clozapine ratio and CYP1A2 CMRs in urine ($r_{urine}=0.734$) and in saliva ($r_{saliva}=0.891$) suggesting a significant involvement of CYP1A2 in the metabolism of clozapine (Spearman correlation coefficient $p<0.05$). High partial negative correlation between clozapine trough serum concentration and CYP1A2 lnCMR in saliva ($r=-0.703$, $p=0.035$) was observed when controlling for age, body mass index and drug daily dose. Smokers exhibited higher mean CYP1A2 CMRs than non-smokers both in urine (8.45±3.46 vs 4.74±2.13, t-test $p=0.043$) and saliva (1.09±0.59 vs 0.46±0.22, t-test $p=0.024$). Statistically significant factors predicting PANSS score, employing linear regression, were serum clozapine concentration ($r=-0.038$, $p=0.031$) and the age of onset of the disease ($r=-3.321$, $p=0.002$).

Conclusion: Increased CYP1A2 activity lowers serum clozapine levels. Smoking, known for its inducing effect on CYP1A2, should be considered when adjusting the dose of clozapine.

Cardiotoxicity in cardiac light chain amyloidosis: implication and therapeutic potential of endoplasmic reticulum stress.

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Purpose: Managing heart failure (HF) in cardiac light chain amyloidosis (AL-CA) is challenging and the gold standard therapies for HF are poorly tolerated or ineffective. The pathophysiological mechanism of light chains' (LCs) cardiotoxicity in AL-CA is elusive. We hypothesized that the comparison of the cardiotoxicity of AL-CA derived LCs with other plasma cell dyscrasias such as multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) would reveal druggable targets to alleviate cardiomyocyte death. We aimed to: 1) identify and biotechnologically produce full-length LCs from AL-CA, MM and MGUS patients or non-clonal LCs from healthy volunteers (HV), 2) investigate LCs' cardiotoxicity and the underlying mechanisms in vitro and 3) identify a target for cardiomyocyte protection. Methods: LCs gene family repertoire was characterized on CD138+ bone marrow cells from patients with AL-CA (n=7), MM (n=2) and MGUS (n=2) and on peripheral blood mononuclear cells from HV (n=2). The LCs' genes were cloned and expressed in Shuffle E. coli cells. LCs' protein folding and amyloidogenic potential were assessed via circular dichroism and electron microscopy respectively. Primary adult ventricular murine cardiomyocytes (pAVMCs) were exposed at various LCs concentrations for cell death evaluation. Cardiotoxicity mechanisms were investigated and led to the evaluation of a therapeutic approach. Results: We successfully identified, produced, and purified the LCs (7 AL-CA, 2 MM, 2 MGUS and 3 HV). The LCs are similar in conformation as beta-sheet and oligomerization. AL-CA derived LCs induce cardiotoxicity in pAVMCs to a different degree that correlates with the extent of amyloid formation. LCs derived from HV, MM and MGUS do not exhibit cardiotoxicity. The common mechanism of the LCs' toxicity includes the increase of endoplasmic reticulum stress (ERS) markers such as Bip and IRE proteins. ERS leads to increased apoptosis in κ-type LCs and to increased autophagy in λ-type LCs. LCs κ-type from patients with AL-CA, MM, and MGUS increase Il-6 mediated inflammation, suggesting that this mechanism is independent of the observed toxicity. Tauroursodeoxycholic acid (TUDCA), a molecular chaperone known to alleviate ERS, abrogates the AL-CA induced cardiotoxicity. Conclusions: AL-CA derived LCs induce cardiotoxicity via ERS which can be alleviated by TUDCA treatment.

Pharmacological manipulation of the GPER1 neuronal membrane estrogen receptor: behavioral effects on male and female rats

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Affective and anxiety disorders represent a significant societal and economic burden and are more prevalent in adult women than men. Recent findings link the newly-discovered G protein-coupled estrogen receptor 1 (GPER1) with depression and/or anxiety and fast neuroestrogen signaling. The distinctive fast-acting antidepressant effect of ketamine also seems to be comparable to the neuroestrogen-mediated GPER1 rapid signaling. We aim to understand GPER1's mechanism of action, which may provide new therapeutic paths for faster and more effective treatment of mood disorders in both sexes. Adult male and female Wistar rats were used and received systemically acute doses of either vehicle, fluoxetine, ketamine, a GPER1 agonist (G1) or antagonist (G15), or a combination of the aforementioned drugs, via intraperitoneal injections. All animals were subjected to the Open field (OF), Light/Dark test (L/D test), Novelty suppressed feeding test (NSFT), and Forced swim test. Initial behavioral results of this ongoing study show that only in male rats the antagonist of GPER1 (G15) tends to inhibit the effects of ketamine on center entries in the OF. Regarding the NSFT, co-administration of ketamine and G15 significantly decreased the delay to consume the presented food compared to ketamine-treated males, indicating an inhibition of ketamine's effect by G15 and overall an anxiolytic effect. Similar to this, G15 negates the impact of G1 on the number of transitions between the two compartments in males during the L/D test, which is indicative of anxiety levels. Notably, GPER1 agonist G1 combined with ketamine exhibit an anxiolytic synergistic effect and decrease the latency to consume the presented food only in males. Moreover, females that received both G1 and KET showed lower activity levels compared to females that received G1 alone. In the present study, co-administration of ketamine and G1 also reduced center entries, indicative of possible anxiogenic effects. Also, ketamine tended to reduce overall activity in female rats, while it had no effect on males. Sex differences in baseline behavioral levels were also identified as before. Findings from this study may lead to the identification and characterization of a novel target, whose pharmacological agonism holds potential as a potential fast-acting antidepressant/anxiolytic.

Ethical Considerations for Phase I Clinical Trials in Greece

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Aim: Phase I Clinical Trials are essential and necessary for drug development and provide initial safety data to support further testing. The aim of this study was to review the guidelines regulating phase I clinical trials in Greece and to illustrate ethical issues that arise during a phase I trial.

Methods: A review of the Nuremberg Code (1947), Declaration of Helsinki (2000), Belmont Report (1979), the EU Regulation No 536/2014 of the European Parliament and of the council of April 2014, the Directive 2001/20/EC, along with national legislation using data from the National Medicines Organization on clinical trials of medicinal products for human use was conducted.

Results: Phase I trials are fundamental on the overall clinical trial process, because they constitute the initiate level for testing a drug or a treatment. The goal of the phase I clinical trial process is to determine initial efficacy of new therapeutic entities, mitigate risks for first-in-human and to define the maximum tolerated dose of the new treatment. The guidelines that regulate phase I include the Nuremberg code, Helsinki Declaration, Belmont Report, Regulation (EU) No 536/2014, the Directive 2001/20/EC, but also national legislation. Weighing benefit – risk prior the initiation and during the clinical trial is significant and necessary to ensure the protection of participants autonomy. The purpose of an Ethics Review Committee is to ensure that risks for clinical trial subjects are responsibly managed and the benefits outweigh the risks. Some of the key ethical issues that arise during a clinical trial phase I are scientific validity, informed consent, protection of the rights and well-being of subjects and fair subject selection.

Discussion : Ethical issues that arise during a clinical trial must be managed effectively, since the primarily purpose is safety and well – being of volunteers. Ethical guidelines and national/eu legislation aim to protect volunteers and to preserve the integrity of the science. The responsible parties for the national legislation for phase I trials in Greece are the National Medicines Organization and especially the Ethics Review Committee that follow the EU legislation without currently implementing any further change.

PEERS — An Open Science "Platform for the Exchange of Experimental Research Standards" in Neuroscience and Biomedical Research

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Due to various and/or conflicting reported outcomes, preclinical research has gotten increasingly complex, leading to a crisis in reproducibility in the field. Without being aware of them, scientists may dismiss critical elements that affect the design and result of their study. An open-access online platform called "PEERS" was created to provide guidance to scientists on which experimental variables and factors are most likely to have an impact on the results of a particular test or model and should be considered during the planning, carrying out, and reporting stages. "PEERS" was constructed by identifying commonly utilised in vivo and in vitro protocols in neuroscience followed by the generation of an extensive list of factors deemed critical for their outcome, based on published literature. Additionally, we created "PEERS," a structured and open system, to rate the quality of the evidence supporting each discovered component and its applicability to a particular approach or model. As a proof-of-concept, specific protocols have so far been chosen for the functioning prototype. Here, we outline the Open Field paradigm in rodents and show how to choose elements unique to the experimental design and the specifics of the scoring system. Finally, we demonstrate how "PEERS" might aid in a community-driven strategy to evaluate evidence and add protocols to the platform. "PEERS" will act as a collaborative exchange and analysis tool to improve data robustness and validity, as well as reproducibility of preclinical research, by assisting scientists in their constructive search for specific factors relevant to their experiments and in the proper reporting of results.

Criminal liability in medical malpractice related to medication

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Abstract: Medication errors may result in adverse effects or even deaths, triggering malpractice civil claims and criminal convictions. Aims of our study were: i. to detect the usual medication missteps, ii. to clarify the elements that establish the criminal culpability for physicians' negligence and, iii. to trace the deadlocks that arise during the criminal investigation of the respective cases and to examine the related causes.

Methods: The research is based on the comparative review of medical and legal literature and the study of the reasoning of recent criminal judgments, in order to distinguish and demonstrate the usual medication errors, to unravel the elements that establish the criminal liability for physicians' negligence and to trace the inefficacies in the investigation of the corresponding criminal cases. Priority is given to highlighting the causes of the observed differentiation between the current research findings of medical science and the corresponding diagnoses in criminal judgments.

Results: An obvious reason for the evidentiary inadequacies in medication-related criminal cases is drugs' indiscernible bond with their adverse effects, as these are often remote and connected to other factors, such as comorbidities, concomitant medication or concurrent surgical failures. Another evenly distinguishable explication is the insufficiencies in the criminal justice system in general, which in many cases impede the immediate implementation of the appropriate investigation and the timely adjudication of the relevant cases. However, a not so distinct, but equally important cause is the disparity between the scientific documentation of the medical acts and the criminal inquiry of their justification.

Conclusions: Beyond the overall improvements that need to take place in both the drug therapy management and the criminal justice system, there are two key changes in the judicial handling of alleged medication misuse incidents that can lead to a more sufficient and fairer investigation of these cases. The first consists in the convergence of the documentation of medical action with the criminal evidence regarding its accuracy and the second in the systematic use of the recently established consensual criminal procedures.

Cell-type and brain-region-specific expression of PLPPRs as a molecular code for developmental neuron morphogenesis in the CNS

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Phospholipid-phosphatase-related proteins (PLPPRs) are a family of neuron-enriched, developmentally expressed membrane proteins that control glutamatergic synapses, filopodia and branch formation, and growth cone navigation. PLPPRs may form heteromeric complexes suggesting diversified effects on bioactive lipid and small GTPase signaling. Studies have focused on hippocampus and cortex early postnatal development, but PLPPRs expression in subcortical brain tissues during development and adulthood is far from known. Furthermore, there are no studies on co-expression of PLPPRs or their neuron-type expression patterns.

Aim: To explore which brain regions and cell types express PLPPRs during development and adulthood.

Materials and Methods: We used quantitative PCR and Western blot for quantifying PLPPR mRNA and PLPPR3 protein expression in 5 tissues and 5 developmental stages. We also developed a custom computational screening tool to mine four publicly available mouse brain single-cell RNA-sequencing datasets (Allen Brain Atlas, mousebrain.org).

Results: Our qPCR analyses suggest ensuing expression of PLPPRs in subcortical brain areas, particularly in structures of the limbic system. Single neuron analysis suggests high PLPPR co-expression in specific adult GABAergic interneuron as well as in cortical and hippocampal glutamatergic subtypes.

Conclusions: PLPPRs are expressed at high levels in the adult limbic system, while GABAergic neurons show the highest degree of PLPPR co-expression. This points to a possible regulatory role of PLPPR heteromeric complexes in GABAergic neuron morphogenesis and function. Lastly, our computational screening approach for single cell sequencing datasets provides a tool to collect information about any gene and neuron type of interest.

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Pharmacological characterization of first-generation catalytic PTEN inhibitors in vitro, in cellulo and in vivo

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Aim: Protein/lipid phosphatases that regulate signal transduction pathways have been largely unexplored towards pharmacological targeting, compared to kinases. We have focused on PTEN, a prominent lipid phosphatase, important for human physiology, that regulates the PI3K/Akt/mTOR signaling pathway and is implicated in several human diseases and pathologies. However, we do not know much about the mechanism of action of PTEN modulators, while targeting PTEN catalytic activity or interaction with membranes, with small molecules, has been proven difficult. In the present study, we have revisited and comprehensively characterized currently used 1st generation PTEN inhibitors, bisperoxo-vanadium (V) complex (bpVs) compounds, focusing on their inhibitory potency and specificity in vitro and in vivo.

Materials-Methods: Several bpVs were synthesized by standard procedures and inhibition of bacterially expressed WT-PTEN was assayed with water-soluble PI(3,4,4)P3 in vitro. Phosphorylation of Akt at Ser473 was used as a proxy marker of PTEN inhibition and activity of the PI3K/Akt pathway in A549 and PC3 cell lines. In vivo, acute or subchronic bpV(phen) administration in Wistar rats was performed and PI3K/Akt pathway activity was analyzed in different tissues. Finally, we investigated bpV(phen) effects on free exploratory behavior and locomotion using the Open Field test.

Results: BpVs exhibit variable inhibition of PTEN activity at μM levels that depends on the reducing conditions in vitro. In cells PTEN inhibition by the bpV compounds results in downstream activation of Akt and mTORC1 in a PTEN-dependent manner. However, bpV compounds increase also Erk1/2 phosphorylation in aPTEN-independent manner. Our results so not confirm the oxidative inhibition of PTEN in cells. In vivo, subchronic administration of bpVs increases pS6 levels in peripheral tissues and suppresses free locomotion of animals in the open field test.

Conclusions: The use of 1st generation PTEN inhibitors, especially bpVs, is widespread, but currently there is no consensus regarding their mechanism of action or safety profile. In our ongoing studies, we are using in vivo/cellular assays to understand the mechanism of action of the already existing PTEN modulators, while our long-term goal is to clarify the importance of pharmacological targeting of PTEN and develop a pattern for the design of new potential drugs.

Do Cardiac Arrhythmias share the same molecular pattern as Seizures? A Bioinformatics Approach

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Aim: Heart and brain are two organs of paramount importance for sustaining human life, and they are both under coordinated regulation of the central autonomic nervous system. Clinicians have previously identified changes in heart rhythm during and after seizures, known as ictal arrhythmias. Epileptic seizures are also associated with alterations in cardiac autonomic function, while arrhythmias have also been proposed to play a role in Sudden Unexpected Death in Epilepsy (SUDEP). There are limited data concerning the burden of arrhythmias in epilepsy and vice versa, while the causes of these conditions are complicated and have severe clinical implications.

As a first step towards understanding the potential mechanisms behind the co-occurrence between Cardiac Arrhythmias and Seizures, we investigated the existence of common expression patterns, using tissue-specific transcriptomic profiles of the two clinical conditions.

Materials and Methods: Two publicly available datasets from the GEO database, one that includes atrial fibrillation (AF) patients and patients with Sinus Rhythm (GSE41177) and one involving patients with febrile seizure (FS) history and patients without (GSE28674) were compared. Samples from the AF dataset derived from atrial tissue, while FS samples derived from the CA3 region of the hippocampus.

Each dataset was analyzed separately for differential gene expression using GEO2R based on statistically significant values (Padj<0.05 and FC>2). The common molecular pattern of the diseases was found using the platform molbiotools.com by creating a Venn Diagram.

Results: In total, 793 overexpressed and 552 underexpressed genes were found in the GSE41177 dataset and 280 overexpressed and 77 underexpressed genes in the GSE28674 dataset. We identified 10 overexpressed and 4 underexpressed genes being common in both datasets. These genes are involved in pathways of the nervous system and cardiac action potential.

Conclusions: In this bioinformatics preliminary study, we uncovered a novel transcriptomic pattern between cardiac arrhythmias and seizures shedding some light into their molecular connection and enabling a deeper understanding of their coexistence.

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Retinal ischemia/reperfusion injury: quest for early molecular biomarkers in a rat pharmacological target evaluation model

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Aim: Ischemia refers to the reduced tissue access to oxygen and metabolic substrates. Tissue reperfusion after an ischemic episode may lead to inflammation and oxidative damage rather than restoration of normal function. Depending on the duration and severity of ischemia, tissue morphology can be altered and necrosis may occur. Retinal ischemia/reperfusion injury (IRI) is a common pathophysiological process in multiple eye diseases including glaucoma, diabetic retinopathy and retinal vascular occlusions. The aim of this study was to develop and characterize an ex vivo model for the evaluation of possible pharmacological targets for the prevention or immediate treatment of IRI.

Methods: Rat retinas were isolated and perfused with artificial cerebrospinal fluid (arti-CSF, 1.2 ml/min). After an initial 60min equilibration period, tissues were perfused in parallel for 332min under normal conditions (95%O2/5%CO2-glucose-arti-CSF; Control), or 266min under normal conditions followed by 66min of ischemic conditions (95%N2/5%CO2-sucrose-arti-CSF; Ischemia), or 26min under normal condition, 66min under ischemic conditions and 240min under normal conditions (IRI). Subsequently, tissues were used either for the immunohistochemical detection of NeuN (ganglion and amacrine cells), GFAP (astrocytes) and GABAergic cells (GABA) and Nissl histochemistry or for the immunoblotting (Dot and Western blot) of cell death, autophagy and ischemia biomarkers. Statistical analysis was performed using the GraphPad Prism software.

Results: Ischemia decreases GABA-immunoreactivity (IR) and inhibits p44/42 and TSC2 activity. Tissue reperfusion for 4h leads to a decrease of retinal nuclear layers cell density and recovery of ischemia-induced decrease of GABA-IR and p44/42 activity. Furthermore, reperfusion results in Akt activation and beclin-1 downregulation. Neither ischemia nor reperfusion had any noticeable effect on NeuN- and GFAP-IR and phospho-p38, AMPK, TNFα, TLR4 and caspase 3 expression at the time points tested.

Conclusions: Retinal reperfusion after 1h of ischemia leads -in a short period of time- to the partial recovery of intracellular GABA levels. Akt activation observed during reperfusion participates, likely, in cell survival and tissue neuroprotection. However, the decreased levels of phospho-p44/42 and phospho-TSC2 during ischemia and the decrease levels of beclin-1 observed during reperfusion argue for a possible early dysregulation of the neuroprotective mechanisms of autophagy and the need for immediate pharmacological targeting.

Study of the anticancer activity of flavonoids derived from kaempferol in pancreatic adenocarcinoma

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Aim: Pancreatic cancer is one of the most lethal types of cancer worldwide. The purpose of this study was to investigate the in vitro anti-cancer activity of flavonoids of kaempferol in pancreatic ductal adenocarcinoma. Specifically, kaempferol and its glycosylated derivatives, tiliroside, and its semisynthetic derivative peracetylated Tiliroside (Tac) were tested for their in vitro anticancer effect against pancreatic cancer cells.
Materials & Methods: The cytotoxic activity of the congeners was tested against the human pancreatic adenocarcinoma cell line PANC-1. To determine the in vitro cytotoxicity the SRB method was implicated. The most active compound, Tac, was further studied for its ability to inhibit the formation of colonies using a colony-forming assay and the migratory ability of PANC-1. Additionally, after exposure of the cells to specific concentrations (10μM and 20μM) and time intervals (6h / 12h / 24h), we examined its effect on the viability of PANC-1 cells via the trypan blue method. Finally, we examined whether Tac is involved in the MAPK pathway by inhibiting the function of p90RSK kinases. To this aim, western blot analysis was performed on these cells to study the expression levels and phosphorylation status of p90RSKs.
Results: Based on the results obtained from the above-described study, it was observed that the peracetylated derivative of Tiliroside, Tac, showed the strongest antiproliferative and cytotoxic effect against the human pancreatic cancer cell line PANC-1. This action was apparently both dose-dependent and time-dependent and from the results of the immunoblotting it that Tac affects the function of p90RSK, by inhibiting some of the most important for the activation of these kinases phosphorylations.
Conclusions: The results indicate significant in vitro anti-cancer activity against the PANC-1 cancer cell line via a dose and time dependent mechanism involving the inhibition of p90RSKs. The mechanism of action of this compound is still under investigation in our laboratory.

Development of novel steroidal and peptidic conjugates for the targeted delivery of cytotoxic agents

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Aim: The synthesis of novel conjugates of cytotoxic agents with steroids and oligopeptides acting as carriers in targeted drug delivery and the evaluation of their anti-cancer activity is reported
Materials and Methods: The cytotoxic agents selected in this work are the DNA alkylating agent POPAM-NH2 which was linked to homo-aza-steroids through an ester bond and the angiogenesis targeting, VEGF inhibitor SRPIN803 which was linked to c(RGDyK) oligopeptide through ether and secondary amide linkers. The toxicity of the synthesized conjugates was evaluated against multiple cancer cell lines and animal models of mice and zebrafish.
Results: The successful synthesis of three POPAM-NH2 and two SRPIN803 conjugates is reported. The steroidal conjugates exhibited strong cytotoxic activity against all tested cell lines (low micromolar IC50 range) while a superior inhibitory effect in tumor growth was also observed in leukemia induced xenografted mice with 2/6 cures. SRPIN803 conjugates demonstrated moderate cytotoxic activity against MCF7 and MRC5 cell lines (mid micromolar IC50 range). Both SRPIN803 and SRPIN803-PEG-c(RGDyK) conjugate demonstrated moderate angiogenesis inhibition in zebrafish animal models.
Conclusions: The conjugation of the cytotoxic agents with carriers that bind selectively to overexpressed receptors in cancer cells is an interesting approach for targeted drug delivery, resulting in reduced side effects and improved anti-cancer activity. Further future research and preclinical studies should reveal the potential of such conjugates in cancer therapy.
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In vitro anticancer activity of σ2 agonist ligands in pancreatic cancer

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Aim: Siramesine, is a σ2 agonist that is reported to show a promising antiproliferative and cytotoxic activity in tumor cells in vitro as well as in vivo. The aim of this study is the investigation of the in vitro activity of siramesine in the human pancreatic cancer cell line PANC-1.
Materials & Methods: We used the methods of SRB cytotoxicity test to determine the GI50, TGI, and LC50 of siramesine against PANC-1, the clonogenic assay, and the wound healing assay to investigate the cytotoxic activity of siramesine, the ability of single cells to make clones, and the ability of the specific cells to migrate when they were exposed to siramesine, respectively. Furthermore, through flow cytometry, we analyzed the viability of siramesine-treated cells and studied the effect of the compound on the cell cycle to investigate if the activity is cell cycle phase-specific.
Results: The data of this study, confirmed that siramesine has a strong antiproliferative and cytotoxic activity under the experimental conditions that have been tested. Moreover, siramesine was found to inhibit the ability of single cells to create colonies and to migrate in a time and dose-dependent manner. The flow cytometry data suggest that siramesine induces cell cycle arrest at the G0/1 phase of the cell cycle.
Conclusions: Siramesine, under the experimental conditions tested herein, exhibits strong anti-clonogenic and anti-migratory activity. Furthermore, we show that the activity of the compound is cell-cycle phase-specific against the PANC-1 cells. These data are reported for the first time worldwide for siramesine. Further studies on the mechanism by which the compound exhibits these effects are ongoing.

Evaluation of the role of a PDK-1 inhibitor in pancreatic cancer

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Aim: This work aimed to study the in vitro anti-cancer activity of the small molecule GSK2334470 which is reported to be a PDK-1 inhibitor, in a human PDAC (pancreatic ductal adenocarcinoma) cancer cell line. PDK1 (Phosphoinositide-dependent kinase-1) is a constitutively Serine/threonine kinase that acts as a master kinase, phosphorylating and activating a subgroup of the AGC family of protein kinases, amongst them interacts with and controls the function of p90RSKs, important downstream kinases of the MAPK/ERK pathway.

Materials & Methods: The effects of GSK2334470 were studied against the PANC-01 cell line. Firstly, was performed an SRB cytotoxicity test of GSK2334470 to determine the in vitro anticancer features of this agent. Subsequently, a clonogenic assay and a wound healing assay were performed to check the inhibitor's antiproliferative ability in both single cells' capability to form colonies and against their migration capacity. Finally, flow cytometry was performed to identify the cell cycle phase in which GSK2334470 acts.

Results: The data obtained from the above experiments suggest that GSK2334470 exhibits good antiproliferative and cytotoxic effects on the pancreatic cancer cell line PACN-01 as its GI50 was found to be around 10μM. In addition, GSK2334470 was both able to inhibit colony formation at the concentration of 0,1μM and the migration capacity of pancreatic cancer in concentrations close to 1μM. Finally, GSK2334470 was observed to act in a phase-specific mechanism as it was found to arrest the cell cycle at the G0/1 to S transition phase.

Conclusions: GSK2334470 PDK-1 inhibitor was found, for the first time worldwide, to exhibit promising in vitro anticancer activity against the PANC-1 human pancreatic cancer cell line which was further found to be time and dose-dependent. This action was found to be also cell cycle phase-specific.

Behavioral and neurobiological evaluation of amphetamine treated and sensitized rats

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Background: Acute amphetamine provokes psychosis-like behavior. Additionally, amphetamine-induced sensitization produces a schizophrenia-related bio-phenotype. Cannabidiol, a non-addictive component of cannabis, has been proposed to display antipsychotic potential. However, studies investigating the impact of cannabidiol on amphetamine-induced hyperactivity demonstrate inconsistent results, while cannabidiol is not adequately tested in amphetamine-sensitization models with relevance to psychosis and schizophrenia. Moreover, the neurochemical underpinnings of cannabidiol's antipsychotic potential are poorly understood.

Aim: This study aims to evaluate the role of cannabidiol on acute amphetamine-treated, and amphetamine-sensitized rats. We focused on the evaluation of behaviors related to positive symptoms and associated dopaminergic alterations in relevant brain regions.

Methods: Adult male Sprague-Dawley rats were treated acutely with cannabidiol or vehicle, followed by amphetamine or saline and assessment of habituated motor activity followed. Another subset of rats received escalating doses of amphetamine or vehicle for 5 days, followed by daily cannabidiol or saline treatment for 5 days. Subsequently, following a challenge dose of amphetamine, their motor activity was recorded. Dopaminergic activity estimations were made in amphetamine-sensitized rats based on measurement of dopamine and its metabolite tissue levels in relevant brain regions, using high-performance liquid chromatography (HPLC). Data were analyzed using two-way analysis of variance (ANOVA) with Bonferroni post-hoc test for multiple comparisons.

Results: Acute amphetamine administration induced robust hyperlocomotion; an effect that was not mitigated by cannabidiol pre-administration. Amphetamine challenge induced overstimulated motor activity in amphetamine-sensitized rats. Interestingly, cannabidiol treatment reversed this effect. Neurochemical analysis of amphetamine-sensitized rats revealed a region-specific dopaminergic profile that was modulated by repeated cannabidiol administration.

Conclusions: Present findings show that cannabidiol does not mitigate acute amphetamine-induced hyperlocomotion, but it counteracts the hyper-responsivity of amphetamine-sensitized rats to an amphetamine challenge. Moreover, cannabidiol modulates the neurochemical dopaminergic profile characterizing the schizophrenia-related bio-phenotype established by escalating amphetamine exposure. These findings provide novel insights with respect to our understanding of amphetamine-induced psychosis and schizophrenia-related bio-phenotypes related to dopamine dysfunction and the antipsychotic potential of cannabidiol.

A proteomic approach to identify platelet-related cardioprotective factors induced by remote ischemic conditioning (RIC) or ticagrelor

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Purpose: Repeated ischemia-reperfusion (IR) cycles in an organ or tissue, remote from the heart (remote ischemic conditioning; RIC) can reduce the extent of myocardial infarction after IR injury. The underlying signal transfer involves humoral factors such as platelets, as we have previously shown that infarct size in rat hearts was reduced by infusion of washed platelets and platelet releasates retrieved after RIC (3×5min blood pressure cuff inflation at 200mmHg on the left upper arm/5 min deflation) performed in healthy volunteers. Also, we have shown that ticagrelor per se induces a humoral cardioprotective signal. The aim of our study was to explore which platelet-related factors contribute to cardioprotection induced by RIC or ticagrelor via proteomic analysis.

Methods: Blood from 18 healthy volunteers (with or without oral pre-treatment of 180 mg ticagrelor) was collected before and 60 min after RIC. In order to examine the effect of RIC or ticagrelor, washed platelets and platelet releasate samples were prepared. Proteome of washed platelets was analyzed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) while platelet releasates were analyzed via GeLC-MS/MS.

Results: 979 proteins were identified across all groups. The PLS-DA analysis proved clear differentiation of the control group versus the examined cardioprotective interventions. In the RIC group, 68 proteins found to be statistically different and were mainly associated with platelet degranulation via the upregulation of myosin light chain kinase (MYLK) and Ca2+/calmodulin-dependent signaling pathways. Proteomic analysis of platelet releasates revealed the upregulation of innate immune system-related pathways, including heat shock protein (HSP)-mediated stress response. The differential expression of HSP70 in platelet releasates obtained after RIC was confirmed by western blot. In addition, 65 proteins in washed platelets from volunteers treated with ticagrelor were differentially expressed, highlighted downregulation of platelet activation pathways and dense granule release, as evidenced by decreased RhoA and Munc13-4 protein expression, respectively.

Conclusion: Our proteomic approach provided a different protein profile of healthy volunteers under the effect of RIC or ticagrelor. RIC and ticagrelor may exert their cardioprotective signal through releasing cardioprotective mediators which may present novel targets in cardioprotection or attenuating platelet activation and aggregatory status, respectively.

Novel H2S-releasing bifunctional antihistamine molecules with improved antipruritic action

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Antihistamines are among the most widely used classes of medicines, being the cornerstone treatment for allergy symptoms; they are also used to reduce pruritus of various etiologies. Hydrogen sulfide (H2S) is an endogenous gasotransmitter with anti-inflammatory actions that also reduces itching. To test whether a combination of an antihistamine with an H2S donor has improved anti-pruritic efficacy, bifunctional molecules composed of a histamine-blocking agent and a H2S-releasing moiety were synthesized and tested in vitro and in vivo. The release of H2S from the hybrid molecules was measured with the methylene blue and lead acetate methods. The inhibition of histamine-induced tissue factor expression by the new compounds was assessed by RT-PCR and was used to prove their ability to block the H1 histamine receptors. All new compounds released H2S in a dose-dependent manner both in solution and when incubated with tissue homogenates or cells. They also retained their histamine blocking activity as assessed by the inhibition of tissue factor expression. Two compounds with the highest potency were evaluated in vivo for their antipruritic, as well as sedative action; they proved to possess higher efficacy in inhibiting histamine-induced pruritus and decreased sedative effects compared to the parent compounds (hydroxyzine and cetirizine), suggesting that they exhibit superior antipruritic action and limited side effects, that likely arise from the H2S-releasing pharmacophore.

Mechanistic links and therapeutic utility of hydrogen sulfide in metabolic syndrome

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Aim: Metabolic syndrome (MetS) is a pathophysiological condition characterized by the coexistence of obesity, insulin resistance, hypertension, and dyslipidemia. Hydrogen sulfide (H2S) is an endogenously produced signaling molecule, involved in several pathways and cardiometabolic diseases. Herein, we determined the role of H2S in the cardiovascular system in a mouse model of MetS. We measured the changes in H2S-producing/degrading enzymes in the heart and aorta. Furthermore, we investigated the role of 3-mercaptopyruvate sulfurtransferase (MPST) deletion in the severity of MetS, a cysteine-catabolizing enzyme that produces sulfide species, and the effects of pharmacological H2S donors in MetS treatment.

Methods: Mice were fed a high fat diet (HFD; 45% of calories from fat) for 15 weeks to induce obesity and hyperglycemia, and received a nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME, 0.5g/L in drinking water), during the last 5 weeks to induce hypertension. Changes in body weight, fasting glucose and glucose tolerance were measured. Blood pressure was determined using a tail cuff system, while mice also underwent echocardiographic assessment of left ventricular (LV) function. The expression of H2S-enzymes was measured by western blot assay.

Results: From the enzymes tested, cystathionine β-synthase (CBS), MPST and thiosulfate sulfurtransferase (TST) were reduced in the aorta, but not the heart, of wild-type mice with MetS. Mice lacking MPST exhibited elevated body weight, greater glucose intolerance and similar degree of blood pressure after HFD/L-NAME administration. Echocardiography analysis showed impairment of LV function in the Mpst-/- mice with MetS, indicating diastolic dysfunction. In addition, we observed that administration of SG1002 (a polysulfide donor) during the last 5 weeks of treatment, ameliorates obesity and glucose metabolism, while administration of GYY4137, a slow releasing H2S donor, additionally exhibited antihypertensive effects.

Conclusion: MetS reduces the levels of H2S-producing and H2S-degrading enzymes in the vasculature. Inactivation of the Mpst gene worsens MetS parameters and results in deterioration of LV function, while pharmacological interventions to restore sulfide levels improve several MetS parameters, indicating that treatment with sulfide donors might be beneficial in patients with MetS.

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A novel method for collection and isolation of spontaneously-released exosomes from mouse and human brain.

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Aim: In the new era of Precision Medicine, extracellular vesicles (EVs), particularly exosomes, exhibit great potential for the diagnosis, prognosis and treatment of brain disorders such as Alzheimer's disease (AD), a complex disease with no effective treatment and poorly understood risk factors. Precision medicine demands high-quality biomarkers, especially for complex brain disorders, where pathological heterogeneity and diverse clinical presentations complicate the development of precise patient-tailored treatments. Thus, the collection and characterization of physiologically relevant exosomes as well as the study of precipitating/risk factors of the disease are of the utmost importance.

Materials and Methods: We present a novel method for collection and isolation of small EVs using multiscale analytical platforms.

Results: In contrast to current approaches for brain exosome isolation that rely on tissue dissociation, which may contaminate the exosome fraction by cellular disruption/damage leading to purer/contaminated EVs yield, we developed a novel method to isolate exosome-enriched EVs from mouse and human brain, relying on their spontaneous release (release method). To confirm the efficacy of the release method and its advantages over the existing, digestion-based approaches, we have presented data of different state-of-the-art and innovative analytical platforms and approaches (e.g., Cryogenic electron microscopy, Nanotracking particle analysis, High-sensitivity flow cytometry, Proteomic analysis, novel ExoView analysis) that helped us to structurally, biochemically and functionally characterize the captured EVs. In addition, we tested the significance of the release method under conditions where biogenesis/secretion of sEVs was pharmacologically manipulated as well as under animal's exposure to chronic stress, a clinically-relevant precipitant of brain pathologies, such as depression and Alzheimer's disease. Our findings show that the released method monitors the drug-evoked inhibition or enhancement of sEVs secretion while chronic stress induces the secretion of brain exosomes accompanied by memory loss and mood deficits suggesting a potential role of sEVs in the brain response to stress and related stress-driven brain pathology.

Conclusions: Overall, the spontaneous release method of sEV yield may contribute to the characterization and biomarker profile of physiologically relevant brain-derived sEVs in brain function and pathology.