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Abstracts from the 15th World Congress on Inflammation

NEW FRONTIERS IN INFLAMMATION: FROM TRANSLATIONAL RESEARCH TO CLINIC

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EDITORIAL

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INFLAMMATION IS AT THE ROOT OF ALL NON-COMMUNICABLE DISEASES

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Inflammation is the root of all degenerative diseases, and its role is appreciated by basic researchers and clinicians. One paradigmatic example is atherosclerosis. For many years, this disease was thought to be the consequence of an excessive accumulation of cholesterol and lipids in the arterial wall. This, of course, is true, but the late Dr. Russel Ross, in a groundbreaking paper published in 1999 called atherosclerosis 'an inflammatory disease' (1). Indeed, evidence builds up and confirms this hypothesis. In 2017 the CANTOS trial reported reduced re-infarction rates in patients given canakinumab, an IL-1β antibody (2, 3). Of note, is the finding that "the lower the better" in terms of reducing inflammation without lowering blood lipoproteins.

Cancer has been termed "the wound that does not heal" (4) and high inflammation is associated with poor outcomes (5, 6). The same line of reasoning holds true for neurodegeneration and its sequelae (7).

In recent times, much of the mortality associated with Covid 19 is due to a 'cytokine storm' and anti-inflammatory therapy is being proven useful (8).

From a pharmacological point of view, it is interesting to note that acute inflammation is quite manageable with the available drugs, be they NSAIDs or corticosteroids. The subtler form of inflammation is the low-grade chronic form. For instance, aging is becoming a public health concern with an important socio-economic dimension. Aging is characterized by an increase in the concentration of inflammatory markers in the bloodstream, a phenomenon that has been termed "inflammaging" (9). The inflammatory response is beneficial as an acute, transient response to harmful conditions, facilitating the repair, turnover and adaptation of many tissues. However, chronic and low-grade features of inflammation might be detrimental to many tissues and normal functions (9). In summary, treating inflammation goes beyond the mere treatment of acute pathologies characterized by pain, swelling, and redness. It is

a great challenge for the entire medical community that requires active pharmacological research.
In this frame, the ***15th World Congress on Inflammation*** **(WCI2022)** is being in Rome for the first time and this issue of PharmAdvances features the abstracts of the many presentations that enrich the meeting.
In conclusion, WCI2022 addresses one of the most important topics in medicine and brings together a top-notch lineup of scientists and clinicians. Perusing this issue of PharmAdvances will certainly help grasp a complete picture of inflammation research and will foster collaborations and innovative investigations.

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ROLE OF ADVANCED GLYCATION END PRODUCTS IN EVOKING SYSTEMIC CHRONIC INFLAMMATION IN ADULT HODGKIN LYMPHOMA SURVIVORS

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OBJECTIVE: current paediatric treatment protocols for Hodgkin lymphoma (HL) include a combination of risk-adapted multiagent chemotherapy together with low-dose involved-field radiotherapy. These therapeutic procedures can trigger an inflammatory response by causing necrosis and tissue injury that stimulate late complications. Hodgkin lymphoma and acute lymphoblastic leukaemia survivors show an increased risk of long-term complications, particularly cardiovascular diseases and malignant neoplasms.
Advanced Glycation End products (AGEs) are formed under hyperglycaemic conditions, but also as a consequence of inflammation and unbalanced oxidative stress, which have been both suggested as a potential determinant of cardiovascular disorders and cancer.
METHODS: 20 HL survivors and 40 age and sex-matched healthy controls were enrolled in the study. After the isolation of peripheral blood mononuclear cells (PBMC) and the collection of plasma, we performed analyses of gene and protein expression by qRT-PCR and Western Blot and the analysis of plasmatic inflammatory and oxidative-stress markers.
RESULTS: survivors showed a condition of higher oxidative stress and an impaired antioxidant status, evaluated in terms of alpha-tocopherol, GSSG/GSH ratio and catalase plasma levels. AGEs plasma levels, expressed as Nε-carboxymethyl-lysine (CML) and methylglyoxal hydroimidazolone (MG-H1), were markedly higher in HL survivors than in healthy subjects. The expression of the receptors for AGEs in PBMC confirmed the dysregulated AGE pathways. These conditions led to the gene and protein over-expression of NLRP3, NFkB and NADPH oxidase in PBMC and, consequently, to an increased plasma levels of C-reactive protein, interleukin(IL)-1β and IL-6.
CONCLUSIONS: in paediatric survivors, the accumulation of AGEs and the subsequent activation of their molecular pathway is associated to the activation of proinflammatory intracellular signalling cascades leading to a chronic low-grade inflammatory response, that persist after the end of anticancer treatments and that may contribute to the onset of late complications.

SAFETY PROFILE OF DRUGS USED IN NON-SMALL-CELL LUNG CANCER: AN ANALYSIS FROM THE ITALIAN PHARMACOVIGILANCE DATABASE

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OBJECTIVE: non-small cell lung cancer (NSCLC) is often caused by molecular alterations that led to the approval of new tyrosine kinase inhibitors (TKIs). Given the clinical relevance of some TKI-related adverse events not yet fully characterized it may be useful to use real-word data to identify new and unexpected adverse drug reactions (ADRs). The aim was to highlight all Italian open data-related ADRs associated with TKIs approved for NSCLC and, consequently, to focus on all regional Sicilian reports.
METHODS: all national publicly-available aggregated ADR reports recorded from 2002 to 2021 into the Reports of Adverse Reactions of Medicines (RAM) system and all complete Sicilian data reported into the Italian spontaneous reporting system (SRS) database having as suspected drugs the following TKIs approved for NSCLC were consulted: afatinib, alectinib, brigatinib, ceritinib, crizotinib, erlotinib, gefitinib, lorlatinib, nintedanib, and osimertinib. Descriptive analyses of national publicly-available aggregated data and full-access regional data were performed to assess demographic characteristics and drug-related variables followed by a more in-depth analysis of all Sicilian ADRs with a case-by-case assessment and a disproportionality analysis of unexpected ADRs.
RESULTS: out of 3,048 collected reports, the 47.5% were related to erlotinib, followed by afatinib, gefitinib, and crizotinib. ADRs were mainly not serious (n = 2,192; 71.9%), related to females (n = 1,592; 52.2%) and to the age group > 65 years (n = 1,617; 53.1%). The most reported ADRs were skin and gastrointestinal disorders (n = 1,766; 57.9% and n = 1,024; 33.6%, respectively) followed by general disorders and infections (n = 536; 17.6% and n = 483; 15.8%, respectively). The case-by-case assessment of Sicilian reports showed 68 serious ADRs (28.8%) with a median time-to-onset of 45 (21-134) days that mainly involved rash (n = 13; 19.1%), diarrhea (n = 10; 14.7%), respiratory failure (n = 7; 10.3%), hypertransaminasemia and dermatitis (both with n = 6; 8.8%), asthenia, folliculitis and nail and nail bed conditions (all with n = 4; 5.9%).
CONCLUSIONS: the reporting of drugs-related ADRs in NSCLC were mostly reported in the literature and not unexpected ADRs were shown. However, further studies are necessary to increase the awareness about the safety profiles of new TKIs onto the market.

EFFECTIVENESS AND SAFETY PROFILE OF BIOLOGICAL THERAPY IN INFLAMMATORY BOWEL DISEASE: REAL LIFE DATA FROM AN ACTIVE PHARMACOVIGILANCE PROJECT

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OBJECTIVE: inflammatory bowel disease (IBD) affects a growing number of people worldwide. With the large use of biological therapies in IBD, post-marketing activities become crucial for

monitoring the long-term safety. The aim was to evaluate the effectiveness and the safety profile of biologics for the treatment of IBD patients during a prospective pharmacovigilance study.
METHODS: from January 2017 to December 2021, all patients with Crohn's Disease (CD) and Ulcerative Colitis (UC) followed by the IBD unit of University Hospital of Messina and treated with at least one biologic agent at the beginning of the study or started a biologic during the study period were enrolled. Demographic, clinical, and disease-related data were collected. Descriptive analyses of patient characteristics at the index date were carried out, followed by an analysis of all adverse events (AEs) and all primary/secondary failures expressed as number of AEs or failures/10 treatment years considering the total years of treatment for each biologic and counting all patients treated with a biologic at least once during the entire follow-up.
RESULTS: out of 675 enrolled patients, 58.1% had CD and 41.9% UC. 58.5% were males and the mean age (± SD) was 44 ± 17 years. The mean disease duration (± SD) was 10 ± 9 years. At the index date, the following treatments were used: 39.9% adalimumab (ADA), 33.3% infliximab (IFX), 21.5% vedolizumab (VED), 2.8% ustekinumab (UST), and 2.5% golimumab (GOL). The total years of treatment were 898 years for ADA, 720 years for IFX, 337 years for VED, 90 years for UST, and 61 years for GOL. Data for AEs and failures were as follows: IFX – 1.3 AEs and 0.8 failures, ADA – 1.0 and 0.9, VED – 1.2 and 1.8, GOL – 1.5 and 3.6, and UST – 2.3 and 1.2. During follow-up, 236 AEs were reported, 28.4% of which were serious mainly in patients treated with UST (0.6), ADA and VED (both 0.5). Infections occurred mainly in patients treated with UST (1.1), skin reactions with ADA and UST (both 0.3), while infusion-related reactions with IFX (0.5). A higher frequency of malignancies was observed in VED-treated patients (0.3%).
CONCLUSIONS: a higher frequency of AEs was noticed for UST, while of failures for GOL and VED, both rarely used as first-line therapies. Moreover, a focus on SAEs, including malignancies, could be highlighted. For this reason, the acquisition of data from clinical practice should be endorsed to better define the safety and effectiveness of new biologic agents in IBD.

THE ANTI-PROLIFERATIVE AND PRO-APOPTOTIC EFFECT OF GENISTEIN IN BRAF^{V600E} WILD-TYPE MELANOMA CELLS ARE MEDIATED BY EGFR DOWN-REGULATION

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OBJECTIVE: melanoma is a very aggressive skin cancer that develops from melanocytes and epidermal growth factor receptor (EGFR) is frequently over-expressed in melanoma, despite its role is still unclear. Genistein is an isoflavone found in soybeans that leads to the inhibition of cell growth and induction of apoptotic cell death in several cancer cell lines by targeting EGFR. The aim of this study is to evaluate how genistein could stimulate apoptosis and reduce cell proliferation in an *in vitro* model of human BRAF^{V600E} wild-type melanoma.
METHODS: the CHL-1 were cultured under standard conditions, seeded in 96-well plates at the density of 10×10^4 cells/well and treated for 24 hours with genistein at doses 6.5, 12.5, 25, 50, 100, 200, 400 µM to evaluate the cytotoxic effect by MTT assay and to determine the IC50. The BRAF^{V600E}-wt melanoma cells were then seeded in 6-well plates and treated with genistein at the IC50 dose of 57 µM. Twenty-four hours after treatment cells were harvested for molecular evaluation, qPCR and Western Blot were performed to evaluate the expression of markers involved in apoptosis (Caspase-3, Bax, BcL- 2) and in EGFR signalling pathway (Ras, Akt).
RESULTS: the MTT test showed that CHL-1 cells exhibited a dose-dependent reduction in viability and the calculated IC50 dose of genistein in CHL-1 cells (57 µM) was selected for the following treatments. Treatment with genistein significantly increased the expression of the pro-apoptotic Bax and Caspase-3, while it reduced the expression of the anti-apoptotic BcL-2, compared to controls. Also, the expression of Akt and Ras significantly decreased in CHL-1 cells treated with genistein compared to controls.
CONCLUSIONS: these data suggest that genistein has a pro-apoptotic and anti-proliferative effect in BRAF^{V600E}-wt melanoma cells, and it probably acts on the EGFR receptor by activating both Akt and MAPK pathways.

INVESTIGATING NEW PATHWAYS OF LIPID CLEARANCE IN PRIMARY AND iPSC-DERIVED FOAM CELLS

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OBJECTIVE: foam cells (FC) are inflammatory, lipid-laden macrophages that drive atherosclerotic plaque formation. They are characterised by excessive accumulation of oxidised low-density lipoprotein (oxLDL) derived lipids that are stored within lysosomes and lipid droplets. The omega-3 polyunsaturated fatty acid (n-3 PUFA), eicosapentaenoic acid (EPA), is an anti-inflammatory lipid mediator reported to have cardio-protective properties, although its mechanism of action is poorly, understood. We investigated the role of EPA in the handling and disposal of oxLDL derived lipids in primary monocyte-derived and induced pluripotent stem cell (iPSC)-derived FC.
METHODS: FCs were generated from $CD14^+CD16^-$ monocytes isolated from human peripheral blood mononuclear cells or differentiated from iPSCs. FC were cultured with dil-labelled oxLDL in the presence or absence of EPA. Treatment with autophagy inducers (triciribine) and inhibitors (chloroquine) were also used to investigate autophagic lipid disposal. Dil-oxLDL uptake was measured by confocal imaging and flow cytometry.
RESULTS: we observed a significant reduction in fluorescent dil-oxLDL signal with EPA treatment, which was detected comparably by confocal imaging or by flow cytometry. This effect was replicated in a model of iPSC-derived foam cell. The inhibitory effect of EPA was mimicked by autophagy inducer triciribine, whilst blocking the autophagy pathway with chloroquine abolished this effect leading to oxLDL uptake.
CONCLUSIONS: we showed that the foam cell phenotype can be reversed in the presence of EPA which may depend on the autophagy pathway to promote lipid clearance. Utilising this avenue for therapeutic intervention may prove beneficial for reducing the incidence of CVD.

EXOGENOUS SIALIDASES AND THEIR ROLE IN THE DEVELOPMENT OF ATHEROSCLEROSIS

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OBJECTIVE: sialidases play an important role in atherosclerosis development due to modification of LDL. The aim of this study was to explore the contribution of viral sialidases to the total sialidase activity in blood plasma. Also, we tested the possibility to change sialylation of lipoproteids in healthy mice upon an injection of immobilized sialidase.
METHODS: the work was carried out in accordance with the principles of good clinical practice on volunteers showing increased sialidase activity of blood plasma in preliminary screening. Immobilized sialidase was injected in healthy mice, animals were sacrificed after certain time and a fraction of lipoproteids was purified for subsequent sialylation determination.
RESULTS: the study of neuraminidase activity during a 6-week clinical study using oseltamivir phosphate was carried out. In 6 volunteers, no significant changes in the sialidase activity of plasma measured immediately after taking the drug, on days 7, 14 and 42, were found.
We found that even a single dose of the immobilized sialidase injected into a mouse reduced the level of Sia in lipoproteids by 50% suggesting that the natural sialidase activity in the murine plasma was low enough for the usage of a higher dosage of the preparation to treat the animals.
CONCLUSIONS: the data obtained indicate the possible absence of the determining role of viral neuraminidases in the appearance of modified low-density lipoproteins (desialylated LDL). A new approach to study the role of sialidase as a proatherogenic factor *in vivo* was established. Research was supported by the Russian Science Foundation (grant#20-15-00264).

ROLE OF STEROL ELEMENT BINDING PROTEIN 1C (SREBP1C) AT THE CROSSROAD BETWEEN TREGULATORY CELL'S FUNCTION AND FATTY ACID METABOLISM

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AIM: cellular metabolism defines T cell polarization and activation; Tregulatory (Treg) cells rely on fatty acids oxidation (FAO) for their suppressive function while glycolysis is preferred for cell migration. We aimed at studying how SREBP1c, a key protein regulating intracellular fatty acid (FA) metabolism, impacts Treg cell metabolism and function.
MATERIALS AND METHODS: a detailed immunophenotyping through flow cytometry and metabolic profiling of isolated Tregulatory ($CD4^+CD25^+$) and in vitro induced Treg (iTreg) cells were performed together with in vitro and in vivo assays of Treg function from SREBP1c KO and WT littermates.
RESULTS: Srebp1c KO mice presented reduced circulating and tissues' level of Treg compared to WT mice (- 66%, $p < 0.01$). Functionally, Srebp1c deficiency was associated with a reduced suppressive (- 21%, $p < 0.01$) and increased migratory function (+ 40%, $p < 0.05$). In Experimental Autoimmune Encephalomyelitis, a model of immune challenge, Treg from KO mice were less effective compared to WT in limiting disease progression. Taking advantage from iTreg we confirmed that the less suppressive and more migratory phenotype was the consequence of Srebp1c deficiency in Treg rather than an effect of reduced cholesterol and triglycerides plasma levels in KO vs WT (- 56%, - 61%, $p < 0.01$). Metabolically, KO iTreg showed an increased glycolytic potential with preserved mitochondrial function. Accumulation of lactate (+ 20%, $p < 0.01$) and increased mTORC1 activation by pS6 phosphorylation (+ 45%, $p < 0.01$) further confirmed a switch to anaerobic glycolysis in KO Treg.
CONCLUSIONS: SREBP1c represents a key player of Treg immunometabolism by controlling glycolysis and cell migration. Metabolic fluxes are ongoing to depict the molecular mechanism/s of this phenotype.

DYNAMICS OF SAA RECEPTORS EXPRESSION IN MOUSE EMBRYONIC FIBROBLASTS AND THEIR SIGNALLING

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OBJECTIVE: serum amyloid A (SAA) is an acute phase protein involved in acute inflammatory response as well as in chronic inflammation and fibrosis.
The aim of this study was to elucidate the expression of its reported receptors TLR2, TLR4 and FPR2 on mouse embryonic fibroblasts (MEF) in inflammatory environment and effects of SAA signalling through them.
METHODS AND RESULTS: MEF were cultured as wild type (WT), TLR2, TLR4 and TLR2/TLR4 KO cells in RPMI with high glucose and stimulated with 100-1000 nM SAA for 24 and 48 hours. While 1-3 fold increase in *TLR2* and *TLR4* expression was observed in SAA-induced WT MEF, the induction of *TLR2* expression was elevated by 50-fold in TLR4 KO following 48 hours of SAA stimulation. 20- 30-fold induction of *TLR2* in *TLR4* KO was observed already at 24 hours. *FPR2* expression in WT, TLR2 and TLR4 KO increased up to 3.5-fold change, however a 15-fold induction in TLR2/TLR4 KO cells at 48h was detected with SAA stimulation.
SAA-induced *IL-6* expression in WT MEF after 24 hours and even more after 48 hours, but this effect was not detected in TLR4 KO or TLR2/TLR4 KO, as confirmed by qPCR and measurement of IL-6 in supernatant. In TLR2 KO, we demonstrated a dose dependent response in *IL-6* expression (up to 150- fold increase). SAA also decreased the expression of a fibrosis marker *ACTA2* in WT MEF, but no effect was observed in TLR2/TLR4 KO.
CONCLUSIONS: we demonstrated dynamic expression of SAA receptors TLR2, TLR4 and FPR2 on MEF, suggesting that cells respond to the lack of one receptor with overexpression of another when stimulated with SAA. We also report distinct effects of SAA receptors in expression of inflammatory and fibrotic genes, emphasizing importance of TLR4 for *IL-6* expression.

RELAXATION OF HUMAN CORONARY ARTERIES, ROLE OF OMEGA-3, RvD1, RvD5 AND MaR1

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OBJECTIVE: imbalanced levels of pro-inflammatory and pro-resolving lipid mediators are associated to the progression of atherosclerosis (Fredman, *et al.* An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat Commun. 2016;7:12859). Previous results of our group showed that mPGES-1 expression and its pro-inflammatory metabolite, prostaglandin (PG)E2, are increased in human atherosclerotic coronary arteries and could be responsible for the vasoconstrictions observed in patients with coronary artery disease (CAD) (Ozen, *et al*. Inhibition of microsomal PGE synthase-1 reduces human vascular tone by increasing PGI2: a safer alternative to COX-2 inhibition. BJP. 2015;174(22):4087-98). Clinical studies have shown a beneficial effect of omega-3 polyunsaturated fatty acids in the regulation of vascular tone in patients with CAD (Daci, *et al*. Effect of omega-3 polyunsaturated fatty acids in modulation of vascular tone under physiological and pathological conditions. Eur J Pharm Sci. 2020;153:105499). We have investigated the role of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and DHA-derived metabolites (RvD1, RvD5 and MaR1) on the vascular tone of human coronary artery (HCA) and the presence of their respective receptors: FPR2/ALX, GPR32 and LGR6.
METHODS: HCA were obtained from patients who underwent heart transplantation or from heart donors. HCA rings were treated during 18 hours with or without EPA, DHA, RvD1, RvD5, MaR1 and placed in a 10 ml organ bath. Concentration-response curves (CRC) were induced by PGE2 or U46619 (TxA2 agonist). Incubation for 30 min with inhibitors of cyclooxygenase (indomethacin 1.7 µM) or NO-synthase (L-NOARG 0.1 mM) was done between two CRC of PGE2.
RESULTS: treatment with DHA (100 µM, n = 5) reduced significantly the contraction induced by PGE2 (CRC: 1 nM-10 µM), while EPA (100 µM, n = 6) showed no effect. Each treatment had no effect on U46619-induced contraction (CRC: 0.1 nM-1 µM, n = 10-11). RvD1, RvD5 and MaR1 (100 nM, n = 12) significantly reduced the PGE2 induced vasoconstriction. Incubation with indomethacin (n = 6-7) didn't modify the PGE2 CRC. While L-NOARG (n = 7) reduced the contractions induced by PGE2 at 0.1 and 1 µM only in RvD1 treated rings. Finally, our immunofluorescence results (n = 3) showed that FPR2/ALX, GPR32 and LGR6 are expressed in HCA endothelial and smooth muscle cells.
CONCLUSIONS: EPA incubation does not affect HCA vasomotricity, whereas DHA treatment decreases only PGE2-induced contraction. DHA-derived metabolites have also the potential to reduce the action of PGE2, independently from prostanoids pathways. We demonstrated that FPR2/ALX, GPR32 and LGR6 receptors are expressed in HCA endothelial and smooth muscle cells. Our results suggest these SPM as a therapeutic approach to reduce coronary artery spasm.

RESOLVIN D1 EPIMER AFFECTS POLYMORPHONUCLEAR CELLS IN A MOUSE MODEL OF PERITONITIS

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OBJECTIVE: Resolvins belong to a family of specialized pro-resolving molecules important for the initiation, self-limitation and resolution of inflammation. Polymorphonuclear cells (PMNs) play a role in the protective inflammatory response, but when infiltrating tissues in excess, they can cause damage and chronic inflammation. In the present study, we investigated the effect of resolvin D1 epimer (EpiRvD1) in resolving peritonitis.
METHODS: peritonitis was induced in 16-week old male C57BL/6J mice by 0.1 % chlorhexidine gluconate (CHX) solution (200 µl i.p. every second day). EpiRvD1 was used as treatment strategy (100 µl i.p. daily at concentration of 100 ng/µl) and control groups for CHX and EpiRvD1 were used (n = 3-4 mice per group). After 7 days of treatment, mice were sacrificed, blood was collected, and abdominal wall tissue was dissociated to obtain single cell suspension. PMNs were determined in the samples by flow cytometry using propidium iodide and antibodies against CD45 and Ly6G.
RESULTS: we observed an increased number of PMNs ($Ly6G^+$) in the CHX peritonitis group (expressed as percentage of live, $CD45^+$ cells), which decreased in both the abdominal wall (30.57 ± 5.51 *vs* 15.50 ± 4.95, p = 0.58) and blood (14.17 ± 0.67 *vs* 8.64 ± 2.26, p = 0.15) in the EpiRvD-treated group.
CONCLUSIONS: daily treatment of peritonitis with EpiRvD1 reduced PMNs both locally in the abdominal wall and systemically in the bloodstream. These results suggest that EpiRvD1 is able not only to reduce infiltration of PMNs to the site of inflammation but also to suppress their development, which may make EpiRvD1 an effective anti-inflammatory therapy.

INFLUENCE OF ENTERIC DOPAMINERGIC PATHWAYS AND INNATE IMMUNITY IN MOUSE AND HUMAN INTESTINAL INFLAMMATION

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OBJECTIVE: innate immunity is involved in ensuring intestinal haemostasis (Caputi V, *et al*. Front Pharmacol. 2017;8:350). Changes in Toll-like receptor 2 (TLR2) and TLR4 signaling as well as in dopaminergic pathways have been shown in inflammatory bowel disease (IBD) patients and experimental in vivo models (Ugalde V, *et al*. Mucosal Immunol. 2021;14:652). Thus, we

aimed to assess TLR4 signaling and dopaminergic machinery in IBD patients as well as in a mouse model of dextran sodium sulphate (DSS)-induced ileitis.
METHODS: TLR2 and TLR4 gene expression was evaluated by qRT-PCR in colon biopsies (CB) obtained from healthy volunteers (N = 3) and matched-IBD patients (N = 3). Male C57/Bl6 and sex- and age-matched TLR4-/- (8 ± 2 weeks old; N = 16 mice/group) received 1.5% DSS in drinking water for 5 days, then switched to regular drinking water for 3 days. TLR4 and TLR2 immunofluorescence was evaluated by confocal microscopy in longitudinal muscle-myenteric plexus whole-mount preparations (LMMPs) from WT mice. Iba1 (a macrophage specific marker), dopamine receptor 1 (D1R), and dopamine transporter (DAT) immunoreactivity were determined in LMMPs by confocal microscopy. mRNA transcripts of inflammatory cytokines IL-1β, TNFα, IL-6 as well as D1R and D2R were evaluated by qRT-PCR. Changes in ileal muscle tension were isometrically recorded following 30 μM dopamine or 30 μM SKF38393 (a D1R agonist) or 30 μM bromocriptine (a D2R agonist).
RESULTS: CB from IBD patients and LMMPs from WT DSS mice showed a significant increase in TLR4 gene expression and immunoreactivity compared to healthy subjects and sham WT mice (+ 30%, $p < 0.05$; + 27%, $p < 0.01$; respectively). In TLR4-/- mice, DSS treatment caused a significant increase of DAT immunoreactivity (+ 27%, $p < 0.01$) together with a significant reduction of D1R (- 18%, $p < 0.05$) and D2R (- 40%, $p < 0.01$) transcript levels, accompanied by increased dopamine-mediated relaxation (+ 20%, $p < 0.01$) that was sensitive to D1R and D2R-mediated activity. A 2-fold reduction of resident Iba1+ macrophages was observed in the LMMPs of TLR4-/- DSS mice with TNF-α, IL-6 and IL-1β mRNA levels comparable to WT sham mice.
CONCLUSIONS: human colitis and mouse ileitis affect TLR4 expression in the enteric nervous system. In DSS-induced ileitis TLR4 signaling influences dopaminergic neurotransmission and ensures macrophage recruitment and inflammatory response. These findings highlight the interplay of TLR4 signaling and dopaminergic machinery in the context of IBD.

SYSTEMIC INFLAMMATION IN ACUTE PANCREATITIS IS MODULATED BY CHANGES IN PROTEINS CARRIED BY CIRCULATING EXOSOMES.

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OBJECTIVE: circulating exosomes released in the early stages of acute pancreatitis showed a different inflammatory capacity that correlates with the ultimate severity of the disease. We evaluated how the final severity of acute pancreatitis correlates with changes in the proteome of circulating exosomes obtained in of plasma of patients in the first 24 hours after hospital admission and explore the role these proteins in the pro-inflammatory activity of exosomes.
METHODS: plasma samples were collected within a year, within 24 hours of admission, from patients with a diagnosis of AP and stored at - 70 °C until used for exosome isolation and proteomic analysis. The final severity of AP was categorized retrospectively as mild, moderately severe or severe. Inflammatory activity of exosomes was evaluated in THP1 cells. The presence of heterodimers S100A8/S100A9 was measured by ELISA. The activation of inflammatory cytokines was evaluated by RT-PCR. Finally, the generation of free radicals and the effect of NADPH-oxidase inhibition was also evaluated.
RESULTS: proteomic analysis of the different exosomes allowed us to identify different groups of proteins, in particular S100A8 and S100A9, whose concentration in exosomes strongly correlated with the clinical classification of pancreatitis. Exosomes from severe acute pancreatitis also induce the activation of NFκB pathway, the expression of IL-1β and TNFα, and the generation of free radicals. These increases could be prevented by the treatment with an NADH-oxidase inhibitor.
CONCLUSIONS: exosomes generated in pancreatitis that will progress to the severe form, showed high inflammatory activity, probably due to the presence of the dimer S100A8/S100A9. This dimer promotes the activation of NADPH-oxidase and the intracellular generation of free radicals, resulting in the activation of the NFκB pathway and the establishment of an inflammatory phenotype in macrophages.

SMOKING CESSATION DOES NOT REVERT SMOKE-INDUCED LUNG INFLAMMATION *IN VIVO*

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OBJECTIVE: cigarette smoke (CS) is widely associated to the establishment of COPD, which is a chronic inflammation-based lung disease. Emerging evidence is demonstrating that the inflammasome may be involved in COPD pathogenesis. Because most of COPD patients are current or former smokers and because the role of inflammasome in COPD is still elusive, the aim of this study is to understand the effects of smoking cessation on smoke-induced lung inflammation.
METHODS: in order to understand the impact of smoking and the inflammasome in COPD, C57Bl/6N mice were exposed to nose-only CS for 16 weeks (smoke group) and for 4 weeks followed by a 16-week cessation period (former group). Control mice, defined as Room Air group, breathed filtered air for the same time.
RESULTS: smoking C57Bl/6N mice had higher enlargement of alveoli, deposition of collagen and mucus production, associated to

the release of IL-1-like cytokines, such as IL-1α and IL-1β at early time points and IL-18 at later time points. Instead, former smokers still presented alveoli enlargement (mean linear intercept, MLI), but lower collagen deposition. Surprisingly, although smoking cessation, lungs of former mice still had high levels of IL-1α, IL-1β, IL-33 and TGF-β. In this scenario, caspase-1 and caspase-11 were in their active form in the lung of former smokers compared to room air-exposed mice.
CONCLUSIONS: in our previous study, we proved that smoking is highly correlated to COPD and lung cancer establishment, in that the activation of the AIM2 inflammasome is at the crossroad between COPD and lung cancer. Here, we demonstrated that smoking cessation does not revert COPD-like features *in vivo*, rather, an IL-1-like inflammatory signature is still evident in the lung. Although the limitation of longer time point than 16 weeks in this study, we believe that the activation of the inflammasome represents a crucial signaling to drive or not the lung towards a chronic inflammatory airway disease, as in the case of COPD and lung cancer.

SUGAR-INDUCED METAFLAMMATION: A COMPARATIVE ANALYSIS BETWEEN DIETARY FRUCTOSE AND GALACTOSE AND PROTECTIVE EFFECTS EVOKED BY PREBIOTIC FRUCTOOLIGOSACCHARIDES IN RATS

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OBJECTIVE: fructose and galactose are among the most consumed carbohydrates, mainly in the form of sucrose, high-fructose corn syrup and lactose. We recently demonstrated that high fructose intake induces the production and accumulation of advanced glycation end products (AGEs), which in turn contributes to the development of a state of low-grade chronic inflammation known as "metaflammation" and lipogenesis in liver and skeletal muscle. The impact of galactose on AGEs accumulation, related metaflammation and *de novo* lipogenesis has not yet been investigated. We thus aimed to investigate, in a strictly controlled *in vivo* environment, the intrinsic ability of the two sugars to exacerbate the deleterious effects of a chronic-fat diet and to identify differences in the activation of inflammatory and lipogenic pathways in target organs of metabolic derangements (mainly liver and skeletal muscle). We further tested the potential efficacy of complex carbohydrates, namely the fermentable dietary fiber fructooligosaccharides (FOS), to counteract these effects.
METHODS: male Sprague-Dawley rats (6/group) were fed 8 weeks as follows: 1) Control 5% fat diet (CNT), 2) 20% fat diet (FAT), 3) FAT + 10% FOS, 4) FAT + 25% galactose (GAL), 5) GAL + 10% FOS, 6) FAT + 25% fructose (FRU) 7) FRU + 10% FOS.
RESULTS: chronic exposure to 20% fat in the presence or absence of simple carbohydrates did not significantly affect body weight gain, blood glucose and lipids or markers of systemic inflammation (TNF-alpha, CRP), whereas an increase in AST and ALT concentrations was detected in both FRU and GAL groups compared to CNT and, most notably, FOS administration counteracted the increase in markers of liver injury. At local level (liver and skeletal muscle) we documented a sugar-induced significant increase in markers of inflammation and lipid impairment and protection evoked by FOS.
CONCLUSIONS: although feeding rats with a diet enriched in both fat and sugars did not result in significant changes at systemic level, we demonstrated that the exposure to fructose or galactose evokes significant changes in the expression of early markers of inflammation and lipogenesis in liver and skeletal muscle, thus confirming the role of simple carbohydrates as main triggers of diet-induced metabolic derangements. The concurrent administration of the prebiotic FOS dampened the sugar-induced local metaflammation and lipid accumulation.

NEUTRALIZATION OF NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (ENAMPT) AMELIORATES EXPERIMENTAL COLITIS: POSSIBLE INTERVENTION ON MACROPHAGE PLASTICITY.

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OBJECTIVE: inflammatory bowel disease (IBD) is a chronic idiopathic disorder in which an accumulation of mucosal macrophages occurs. Nicotinamide phosphoribosyltrasferase (NAMPT) has been postulated as a novel target. Cellular NAMPT is present in two different forms: intracellular NAMPT, involved in NAD synthesis (Chiarugi *et al.*, 2012), and an extracellular form, eNAMPT that acts as cytokine on immune cells, binding to a still unknown receptor (Camp *et al.*, 2015). eNAMPT could be a mediator of some macrophage- related activity, as a pro-inflammatory stimulus. The aim of our work was to target eNAMPT thanks to a novel anti- eNAMPT antibody (C269, Colombo *et al.*, 2020).
METHODS: the murine DNBS-model was used to emulate IBD disease *in vivo*, administering C269 or recombinant eNAMPT. Moreover, we used peritoneal macrophages (PECs) extracted from mice peritoneum, after thioglicollate induction.
RESULTS: exogenous administration of eNAMPT in DNBS model determined a worsening of IBD symptoms. These symptoms are reduced after the treatment with the anti-eNAMPT antibody, and a down- expression in proinflammatory IFNγ-associated genes. Moreover, C269 reduced the frequency of myeloid and T cells in *lamina propria*.
Ex vivo data on PECs reveals that eNAMPT has pro-inflammatory properties. We have demonstrated that eNAMPT priming of PECs enhances IFNγ-dependent response, through STAT and NF-κB-dependent mechanism, reverted with C269 pre-treatment.
CONCLUSIONS: taken together, our data demonstrated that eNAMPT exacerbates DNBS-associated symptoms, in which its neutralization could ameliorate the pathogenesis of the disease, focusing on macrophages plasticity as a possible target, prompting anti-eNAMPT antibody as a possible treatment in IBD.

INVOLVEMENT OF CYTOKINES IN HEART FAILURE-ASSOCIATED KIDNEY DYSFUNCTION: A FOCUS ON THE RENAL ION CHANNELS

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OBJECTIVE: heart failure (HF) often coexists with various comorbidities of which declining renal function is of particular importance. The interaction between heart and kidney in this setting is complex, involving also activation of pro-inflammatory systems. Renal ion channels (ClC-K, Kir4.1, Kir5.1), play a key role for salt reabsorption and may represent targets in HF management. Indeed, T cells interaction with nephron, leading to chloride efflux by Kir4.1-ClC-K pathway, contributes to hypertension development. To investigate the inflammatory pathways involved in HF-associated kidney dysfunction focusing on renal ion channels, we performed an analysis of ion channels expression and activity by *in vivo/in vitro* studies.
METHODS: we used Dahl salt-sensitive (SS) hypertensive rats for characterizing renal ion channels expression by molecular biology. Ion channels function was tested through heterologous expression and patch clamp analysis.
RESULTS: we detected a significant reduction of ClC-K1, Kir4.1 and Kir5.1 mRNA in kidney of Dahl/SS rats *vs* control rats. A ClC-5 mRNA decrease, a Cl^-/H^+ exchanger involved in protein reabsorption, was also observed. Dahl/SS rats showed high TNF-α, IL-6, TGF-β plasma levels. We are currently evaluating the effects of cytokines on heterologously expressed renal ion channels. Preliminary results indicate that TNF-α incubation affects the activity of ClC-Kb channels.
CONCLUSIONS: reduced expression of ClC-K1, Kir and ClC-5 in Dahl/SS rats could be related to salt wasting and proteinuria observed in HF in relation with cytokines overproduction and the modulation of ion channels could impact HF disease management.

POTENTIAL EFFECTS OF A SYNTHETIC FPR2 AGONIST ON NEURO-INFLAMMATION IN TWO MOUSE MODEL OF AUTISM SPECTRUM DISORDERS

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OBJECTIVE: Autism Spectrum Disorder (ASD) is a lifelong neurodevelopmental disorder characterized by repetitive behaviors and the impairment of social abilities. Mounting evidences reported that one of the most common risk environmental factor associated with ASD is the ongoing inflammation (Depino AM. Peripheral and central inflammation in autism spectrum disorders. Mol Cell Neurosci. 2013;53:69-76). Among lipoxins, lipoxin A4 (LXA4) is one of arachidonic acid metabolites with potent anti-inflammatory properties mediated by its receptor formyl peptide receptor 2 (FPR2). The role of this receptor in ASD is poorly investigated; only low levels of LXA4 have been found in the plasma of children with autism (Yan CL, *et al.* Decreased plasma levels of lipoxin A4 in children with autism spectrum disorders. Neuroreport. 2015;26(6):341-5).We investigated the role of FPR2 on neuroinflammation in ASD through the use of a novel synthetic agonist.
METHODS: for our studies we used two different mouse models of ASD, BTBR mouse strain and mice prenatally exposed to valproic acid. We tested different doses of the compound (1, 5, 10 and 50 mg/kg) intraperitoneally injected in acute (2 hrs) or chronic regimen (4, 8, 15 days). Then, mice were subjected to several behavioral tests and ex vivo analyses to investigate the role of inflammation associated with ASD.
RESULTS: our results demonstrated the effect of a synthetic ligand of FPR2 receptor, at the active dose of 10mg/kg intraperitoneally injected for 8 consecutive days, on the hippocampal neuro-inflammatory profile of both ASD mouse models, such as the inhibition of the production of pro-inflammatory cytokines, as well as on the modulation of hippocampal expression level of LXA4 and FPR2. These findings were accompanied by a significant positive effect on social behavioural tests in both ASD mouse models. Moreover, preliminary in vitro studies, by using primary cultures of hippocampal neurons, revealed a restored the neurite outgrowth of BTBR neurons after the treatment.
CONCLUSIONS: considering the crucial role of inflammation in ASD, acting on central inflammation could be an important therapeutic approach to obtain an optimal management of ASD.

SPINAL CORD STIMULATION ACTS ON INFLAMMATORY STATE AND IMPROVES QUALITY OF LIFE OF PATIENTS WITH CHRONIC PAIN

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OBJECTIVE: chronic pain is one of most disabling condition that strong influences quality of life; its exacerbation appears to be sustained by a chronic neuro-inflammation state. Spinal Cord Stimulator (SCS) implant represents the last chance for patients with chronic pain. There is a high inter- individual variability in term of success of treatment and the long-term pain relief (Turner JA, *et al.* Spinal cord stimulation for patients with failed back surgery syndrome: a systematic review of effectiveness and complication. Pain 2004;108(1):137-47). Different factors could be influence SCS outcome, including psychological state. Moreover, it is recently supposed that chronic pain shares some mechanisms with frailty, influencing each other (Saraiva MD, *et al.* Persistent pain is a risk factor for frailty: a systematic review and meta-analysis from prospective longitudinal studies. Age Ageing. 2018;47(6):785-93). Therefore, frailty could negatively impact on SCS benefit or, conversely, a pain relief could improved frailty symptoms. The aim of this study is investigating the possible correlation between some frailty related conditions and chronic pain; evaluate SCS implant impact on inflammatory state and quality of life in chronic pain population.
METHODS: patients with chronic low back pain, eligible for SCS implant, were enrolled and blood samples were collected at baseline and 1-3-6 months after implantation. Some frailty related symptoms, including psychometric parameters were investigated at each follow up. Finally, quality of life was evaluated before and after implant. The expression of pro- and anti-inflammatory cytokines mRNAs in PBMC was determined by real-time PCR. The analysis on cytokines released by PBMCs differently stimulated is also ongoing.
RESULTS: our preliminary results show that SCS significantly improves mood alterations and pain relief, improving quality of life of patients. A reduction of gene expression of some inflammato-

ry biomarkers was shown. Pain severity and decrement of some frailty-related conditions was also found to be correlated.
CONCLUSIONS: it appears that frailty and pain could influence each other, and successful pain treatment may reduce progression of symptoms related to frailty. SCS shows to play an important role on inflammatory state and significantly improves patients' quality of life. It is need investigate if these improvements remain a long term.

NEUTROPHIL EXTRACELLULAR TRAPS TRIGGER AN ENHANCED PRO-INFLAMMATORY RESPONSE IN MACROPHAGE SUBPOPULATIONS IN RHEUMATOID ARTHRITIS PATIENTS

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OBJECTIVE: activated neutrophils (PMN) form neutrophil extracellular traps (NET), fibers of DNA and associated proteins expelled in the extracellular space. Increased NET formation has been reported in rheumatoid arthritis (RA). Moreover, macrophages play a key role in RA pathogenesis. We have previously shown that NETs are pro-inflammatory on resting non-polarized macrophages, whereas NET partly inhibit the response of LPS-stimulated macrophages (Ribon MJ, *et al.* Neutrophil extracellular traps exert both pro- and anti-inflammatory actions in rheumatoid arthritis that are modulated by C1q and LL-37. J Autoimmun. 2019;98:122-31). We aimed to characterize inflammatory properties of NET on polarized macrophage sub-populations in RA and healthy donors (HD) and to determine the mechanisms and pathways involved.
METHODS: primary blood PMN/monocytes were freshly purified from HD and RA patients. Monocytes were differentiated into non-polarized (M0), anti-inflammatory (M2a, M2c) or pro-inflammatory (M1) macrophages with cytokines. NETs were induced *in vitro* by stimulating PMN with PMA and characterized. Mouse (wild-type, TLR9-deficient or C1q-deficient) bone marrow cells were used to differentiate macrophages and to prepare NET from purified PMN. Macrophages were cultured with NET in the presence/absence of LPS. Cell purity, phenotype and activation were estimated by flow cytometry. Cytokine secretion was measured by ELISA. The pathway triggered was estimated by bulk RNA-seq.
RESULTS: all resting macrophage subpopulations were activated by NET, leading to a pro-inflammatory response. The pro-inflammatory cytokine IL-8 was secreted, whereas secretion of the immunomodulatory IL-10 was minimal. Even M2 macrophages were activated toward this pro-inflammatory profile, with a stronger response in RA patients. In response to LPS, NET inhibit IL-6 secretion in HD macrophages whereas RA M1 and M2a macrophages were resistant to this anti-inflammatory activity of NET. Moreover, RA M1 macrophages produce more IL-8. In mouse macrophages, TLR9 is not involved in NET recognition. Moreover, C1q loaded on NET is not necessary to trigger macrophage activation. RNA-sequencing confirmed the induction of pro-inflammatory mediators, whereas IL-10 was down-regulated, and suggested that the aryl hydrocarbon receptor (AHR) pathway is involved in macrophages in response to NET.
CONCLUSIONS: pro-inflammatory activities of NET clearly dominate anti-inflammatory activities in macrophages, particularly in RA patients, suggesting a pathogenic role of NET in RA by inducing a pro-inflammatory response of macrophages through AHR.

INTESTINAL WOUND HEALING DELAY IN INFLAMMATORY BOWEL DISEASES, INVOLVEMENT OF EPITHELIAL ELASTASE-2

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OBJECTIVE: intestinal wound healing is an essential step in achieving clinical remission of patients with Inflammatory Bowel Disease (IBD). We demonstrated that the intestinal epithelium produces and secretes a protease named elastase-2 (ELA2). In IBD patients, ELA2 epithelial expression is significantly enhanced both in inflamed and non-inflamed areas. We have generated conditional transgenic mice ELA2 (pVillin-CreERT2-hELA2) and this overexpression is sufficient to induce a leaky barrier, as observed in IBD. We hypothesized that ELA2 overexpression maintains epithelial dysfunctions and participates in delayed wound healing.
METHODS: to reveal the effects of ELA2 overexpression on intestinal wound healing defects, colitis was chemically induced (2.5% of DSS) for 7 days, followed by 7 or 14 days without DSS. Mucosal integrity was assessed *in vivo* using an endoscopic procedure. Histological damage scores were also evaluated. The expression of more than 25 markers of epithelial repair was quantified.
RESULTS: after 14 days of repair, ELA2 hyperactivity impaired body weight recovery and Tg-ELA2 mice still showed erythema and granular colonic mucosa. The presence of infiltrated immune cells, a decrease in the number of goblet cells and a thinner mucus layer in Tg-ELA2 mice confirmed the delay of healing. The re-epithelialization process was also impaired, as demonstrated by the decreased expression of *CD74*, *Egf, Annexin A2* and *Muc2* genes in Tg- ELA2.
CONCLUSIONS: ELA2 hyperactivity seems to alter repair mechanisms of the epithelial barrier. Altogether, our data suggest that ELA2 could constitute an interesting molecular target to improve intestinal wound healing in IBD patients.

miR-146a MODULATES TLR1/2 AND 4 INDUCED INFLAMMATION AND LINKS IT WITH PROLIFERATION AND LIPID PRODUCTION VIA THE INDIRECT REGULATION OF GNG7 IN HUMAN SZ95 SEBOCYTES

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BACKGROUND: activation of Toll-like receptors (TLR) 1/2 and 4 are central in inducing inflammation in sebocytes by regulating the expression of protein-coding mRNAs, however the microRNA (miRNA) profile in response to TLR activation and thus the possible role of miRNAs in modulating sebocyte functions has not been elucidated (Kim J, *et al*. Activation of Toll-Like Receptor 2 in Acne Triggers Inflammatory Cytokine Responses. J Immunol. 2002;169:1535-41).
METHODS: we determined the microRNA profile after TLR activation of SZ95 human sebocytes by miRNA sequencing. With chromogenic in situ hybridization on paraffin-embedded human tissue samples, we detected miR-146a *in vivo*, while using the SZ95 immortalized sebaceous gland cell line we determined the gene expression profile and changes in lipid synthesis and cell proliferation 72 h after treatment with miR-146a siRNA or mimic. By using migration assay we also determined the chemoattractant potential of sebocytes transfected with miR-146a inhibitor or mimic sequences. We examined IL-8 secretion of transfected sebocytes by using ELISA. To obtain global transcriptome data on miR-146 regulatory role in SZ95 sebocytes, high throughput mRNA sequencing analysis was performed on Illumina sequencing platform.
Results: in this work, we identified miR-146a to have the highest induction in the TLR1/2 and 4 activated SZ95 sebocytes and found that its increased levels led to the down-regulation of IL-8 secretion, decreased the chemoattractant potential, and stimulated the proliferation of sebocytes. Assessing the gene expression profile of SZ95 sebocytes treated with a miR-146a inhibitor, the induction of *GNG7* was one of the highest, while when cells were treated with a miR-146a mimic, the expression of *GNG7* was down-regulated. These findings correlated with our *in situ* hybridization results, that compared with control, miR-146a showed an increased, while *GNG7* a decreased expression in sebaceous glands of acne samples. Further studies revealed, that when inhibiting the levels of *GNG7* in SZ95 sebocytes, cells increased their lipid content and decreased their proliferation (Törőcsik D, *et al*. Genome wide analysis of TLR1/2- and TLR4-activated SZ95 sebocytes reveals a complex immune-competence and identifies serum amyloid A as a marker for activated sebaceous glands. PLoS One. 2018;13).
CONCLUSIONS: our findings suggest that miR-146a could be a potential player in acne pathogenesis by regulating inflammation, inducing proliferation, and through the indirect down-regulation of *GNG7*, promoting the lipid production of sebocytes (Lovászi M, *et al.* Sebum lipids influence macrophage polarization and activation. Br J Dermatol. 2017;177:1671-82).

DEFICIENCY OF TOLL-LIKE RECEPTOR 4 SIGNALING IN SMALL INTESTINE NEUROMUSCULAR DYSFUNCTIONS DURING AGEING

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OBJECTIVE: neurodegeneration increases substantially during ageing leading to higher prevalence of cognitive decline, gastrointestinal disorders and infection for impaired immunity. Thus, we aimed to assess the effect of TLR4 deficiency on ENS homeostasis during ageing.
METHODS: confocal immunoreactivity of the neuronal proteins HuC/D and alpha-synuclein as well as the glial markers S100β and GFAP was determined in longitudinal-muscle-myenteric plexus whole-mounts preparations (LMMPs) of male young (aged 3 ± 1 months) and middle adult (aged 12 ± 1 months) C57/Bl6 (WT) and sex- and age-matched TLR4-/- mice (N = 16 animals/group). Pro-inflammatory IL-18 levels were measured in brain and ileal samples by qRT-PCR. Changes in ileal contractility were recorded following: i) cumulative addition of carbachol (CCh; 0.1-100 μM); ii) electric field stimulation (EFS, 0-40 Hz); iii) 10 Hz-electric-field-stimulation in non-adrenergic, non-cholinergic (NANC) conditions (1 μM atropine+1 μM guanethidine), with or without 0.1 μM 1400W (inhibitor of inducible nitric oxide synthase, iNOS), or 100 μM L-NAME (pan-NOS inhibitor).
RESULTS: in brain and ileum samples from aged mice, a 3-fold increase of IL-18 mRNA levels was found only in older WT mice. In 12-months-old WT mice, reduced cholinergic receptor- mediated response (Emax = - 43%; $p < 0.001$) together with a defective neurotransmission (- 45% at 10 Hz; $p < 0.001$) was found compared to young mice. In TLR4-/- mice, ageing determined increased EFS-mediated response (+ 40% at 10 Hz; $p < 0.05$) with no changes in CCh-induced contraction. An age-related significant increase in NANC relaxation resulted to be increased and sensitive to 1400W or L-NAME in both 12-months-old WT and TLR4-/- mice. A significant reduction of the total number of HuC/D+ neurons (- 26%; $p < 0.01$) associated to increased S100β (+ 25%; $p < 0.001$), GFAP (+ 28%; $p < 0.05$) and alpha-synuclein (+ 96%; $p < 0.01$) immunoreactivity, was found in LMMPs of aged WT mice with no alterations in myenteric neuroglial network of 12-months-old TLR4-/- mice.
CONCLUSIONS: during ageing TLR4 signaling influences CNS and ENS inflammatory status and small intestine dysmotility and myenteric neuro-glial plasticity. These findings uncover the modulatory role of TLR4 in the gut-brain pathways affected by senescence that could be targeted for promoting healthy ageing.

HYPER-INFLAMMATION AND SURVIVAL OF SEPTIC MICE ARE IMPROVED THROUGH PHARMACOLOGICAL INHIBITION OF THE FAK-PyK2 AXIS

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OBJECTIVE: sepsis is characterized as a hyper-inflammatory state that contributes to organ failure and mortality, being considered one of the major public health concerns. Recent findings suggest a potential role of two non-receptor proteins tyrosine kinase, the Focal adhesion kinase (FAK) and Proline-rich tyrosine kinase 2 (Pyk2) in mediating inflammation in diseases such as endometriosis, cancer, atherosclerosis and asthma. Therefore, our study aimed to investigate the role of FAK-PyK2 during sepsis and the potential beneficial effects of the pharmacological modulation of the FAK-Pyk2 pathway by administering a potent reversible dual inhibitor of both FAK and Pyk2, PF562271 (PF271) to septic mice.
METHODS: sepsis was induced by cecal ligation and puncture (CLP) procedure in five-month-old male C57BL/6 mice. One hour after the CLP or Sham procedure, mice were randomly assigned to receive PF271 (25 mg/kg, s.c.) or vehicle. Organs (liver and kidney) and plasma were collected 24h after surgery for analyses. In another group of mice, survival rate was assessed every 12h over the subsequent 5 days.
RESULTS: twenty-four hours after CLP, experimental sepsis led to a systemic cytokines storm including both pro-inflammatory cytokines (TNF-a, IL-1B, IL-17 and IL-6) and the anti-inflammatory cytokine IL-10. The systemic hyperinflammatory state was accompanied by high levels of plasma markers of organ damage such as ALT, AST, Creatinine and lactate, as well as a high severity score. All parameters were reversed through treatment with PF271. We then mechanistically demonstrated that experimental sepsis induced a local overactivation (liver and kidney) of FAK and Pyk2, which paralleled via p38 MAPK the expression/activation of the NLRP3 inflammasome, adhesion molecules (ICAM-1, VCAM-1 and E-selectin), NOS-2 and Myeloperoxidase. Treatment with PF271 reduced the activation of FAK-Pyk2 and consequently abolished the inflammatory abnormalities orchestrated by sepsis. Finally, survival analysis revealed that PF271 significantly prolonged the survival time of septic mice.
CONCLUSIONS: in summary, our data suggest for the first time that, at least in part, inflammation is driven by the FAK-Pyk2 pathway and its pharmacological modulation may represent a new strategy for the treatment of sepsis, due to its potential effects in counteracting the hyperinflammatory state, which in turn reduces organ damage and ultimately promotes long-term survival protection.

CALCIUM SUPPLEMENTATION IMPROVES MUSCLE FUNCTION AND IRISIN IN MICE

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OBJECTIVE: physical training and natural diet can change the expression of myokines and improve muscle function. Irisin is known to be produced during training and has anti-inflammatory and pro-metabolic effects on muscle and adipose tissue.
METHODS: in this study mice subjected to 14 days of training sessions and receiving a calcium rich diet were tested.
RESULTS: at the end of the study force was improved and fatigue reduced in mice taking calcium compared to controls. Additionally an enlargement in muscle fibers was observed together with increased circulating levels of irisin. AMPK as well as Pec-1a and Pparg levels were also increased in mice taking the calcium rich diet compared to controls. Furthermore muscles of mice taking calcium rich diet demonstrated increased levels of nuclear respiratory factor 1 (NRF1), mitochondrial transcription factor A (TFAM), glucose transporter 4 (GLUT4) and mitochondrial uncoupling protein 3 (UCP3) leading to increased mitochondrial biogenesis.
CONCLUSIONS: these results suggest that calcium rich diet together with exercise might improve muscle strength and metabolism, reducing inflammatory factors.

OSTEOREGULATORY PEPTIDE REPAIRS BONE IN HEALTH AND AGE RELATED MUSCULOSKELETAL DISEASES

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OBJECTIVE: current treatments for age-related and inflammatory musculoskeletal (MSK) diseases aim to prevent further bone loss or treat the underlying processes driving inflammation. However, few drugs specifically stimulate healthy bone formation to aid bone repair. This study investigates the effect of an endogenous 14-amino acid peptide (PEPITEM) and sphingosine-1-phosphate (S1P) on bone remodelling.
METHODS: mice were treated with PEPITEM or PBS for two weeks. Micro-CT, 3-point bend testing and tartrate-resistant acid phosphatase (TRAP) staining were used to assess changes in trabecular parameters, bone strength and osteoclast counts. Quantification of osteoblast or osteoclast activity *in vitro* were performed using alizarin red staining, alkaline phosphatase ELISA, TRAP staining or RNAseq analysis.
RESULTS: PEPITEM therapy increased bone volume:trabecular volume ratio, limb stiffness and failure force load when compared to control mice. PEPITEM therapy also halted further bone loss in ovariectomised and arthritic mice. *In vitro,* PEPITEM treatment induced osteoblast differentiation and mineralisation, whilst having no effect on cultured osteoclasts. Conditioned media from PEPITEM treated osteoblasts or exogenous S1P reduced osteoclast number *in vitro*. Importantly, genes involved in S1P synthesis and detection were detected in cultured osteoblasts and osteoclasts using RNAseq. Moreover, S1P treatment promoted expression of inflammation-related genes (*e.g.*, cytokine signalling) in osteoblasts.
CONCLUSIONS: PEPITEM acts as an endogenous pro-anabolic factor promoting osteoblast mineralisation and bone growth in health and disease. Crucially osteoblasts release an anti-osteoclastogenic molecule, potentially S1P, when treated with PEPITEM further limiting bone resorption. Thus, PEPITEM offers a potential treatment for MSK diseases that cause osteolysis and impaired bone formation.

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ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECT OF LYCOPENE TO BE USED FOR THE TREATMENT OF OSTEOPOROSIS

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OBJECTIVE: osteoporosis is defined as a skeletal disorder characterized by decreased density of normally mineralized bone, consequently bone fragility occurs mainly due to aging with an alteration of the dynamic balance of bone remodeling, with increased osteoclasts activity and reduce bone formation. The pathogenesis of osteoporosis is related to oxidative stress that increased with aging or in an inflammatory state, in fact, reactive oxygen species (ROS) suppress osteoblast differentiation while promote osteoclast activity. Thus anti- oxidant compounds might have a role in reducing bone loss, to this end we tested lycopene, a carotenoid that inhibits the activation of NF-κB and the release of pro-inflammatory cytokines in an experimental model of osteoblast impairment due to H2O2 stimulation.
METHODS: human fetal osteoblasts hFOB 1.19 (ATCC® CRL-11372™) were cultured under standard conditions and were stimulated with H2O2 at 300 μM for 6 hours, later on lycopene was added at different doses (0.5, 1 and 2 μM) for up to 24 hours. At the end of the experiment qRT-PCR and Western Blot were performed to evaluate the expression of Nrf2 and pro-inflammatory cytokines.
RESULTS: results demonstrated that the expression of Nrf2 is increased using lycopene as a result of an antioxidant mechanism; H2O2 increased TNF-α, IL-1β, and IL-6 levels, while lycopene inhibited the H2O2- induced production of these pro-inflammatory cytokines.
CONCLUSIONS: these preliminary data suggest that lycopene, a component of Mediterranean diet, could be used to reduce the inflammation and the oxidative stress related to osteoporosis.

CAPE, 6-MSITC AND PQM130 ARE ALL ABLE TO REDUCE NEUROINFLAMMATION AND COGNITIVE DECLINE IN A MOUSE MODEL OF AD. IS ALZHEIMER'S DISEASE TRIGGERED BY NEUROINFLAMMATION?

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OBJECTIVE: neurodegeneration defines a set of pathological conditions characterized by the progressive and consistent loss of Central Nervous system functions. Alzheimer's disease (AD) is the most common neurodegenerative disease in older people. Because of the complex nature of AD, a combined pharmacological approach is needed to control neurodegenerative processes and manage the main symptoms. Donepezil is the first-line acetylcholinesterase inhibitor used for AD treatment. Although several studies have demonstrated the symptomatic efficacy of donepezil treatment in AD patients, the possible effects of donepezil on the AD process are not yet known. The experimental activity has been oriented to evaluate and characterize molecular and cellular mechanisms that contribute to neurodegeneration induced by the Aβ oligomers and potential anti-inflammatory and neuroprotective effects of different compounds such as Caffeic Acid Phenethyl Ester (CAPE), 6-(Methylsulfinyl)hexyl Isothiocyanate 6-MSITC and Feruloyl-Donepezil Hybrid Compound (PQM130).
METHODS: the neuroprotective effects of CAPE (10mg/kg), 6-MSITC (5 mg/kg) and PQM130 (0.5-1 mg/kg) were examined in a murine AD model, obtained by intracerebroventricular (i.c.v.) injection of Aβ1-42 oligomers (Aβ1-42O). The treatment started 1 h after the surgery for the next 10 days. At the end of the treatment half of the groups were sacrificed to proceed with biomolecular analysis while the other animals underwent behavioral assessment before the sacrifice.
RESULTS: an intracerebroventricular (i.c.v.) injection of AβO into the mouse brain increased reactive oxygen species levels, neurodegeneration, neuroinflammation, and memory impairment. In contrast, the intraperitoneal administration of CAPE, 6-MSITC and PQM130 after i.c.v. AβO- injection reduce memory impairments, reactive oxygen species in hippocampal tissues and interfere positively with Nrf2-pathway. Moreover, activation of caspases, increase of inflammatory factors such as GFAP and Iba-1 were inhibited by our three compounds.
CONCLUSIONS: our findings highlighted that CAPE, 6-MSITC and PQM130 are potent multi- functional agent against AD and could act as promising neuroprotective and anti-inflammatory compounds.

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SODIUM PENTABORATE PENTAHYDRATE PREVENTS LPS-INDUCED NEUROINFLAMMATION IN MICROGLIAL CELLS

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OBJECTIVE: the trace mineral boron is an essential plant micronutrient but its potential role on human health has been recently recognized. Boron was shown to have anti-inflammatory, antioxidant, anti-cancer properties as well as cognitive performance enhancing effects. Sodium pentaborate pentahydrate (NaB) is the less-likely toxic boron-based compound. Although limited studies are suggesting the importance of boron derivatives in inflammation, the precise mechanisms of action are not yet well elucidated. The aim of this study is to investigate the prophylactic anti-inflammatory effect of NaB in lipopolysaccharide (LPS)-stimulated human microglial clone 3 cell line, HMC3.
METHODS: HMC3 cells were treated with different concentrations of LPS (0.05-10 μg/mL) to select the non-toxic dose that induces inflammation. Also, cells were treated with various NaB concentrations (62.5-1000 μg/mL) to determine the non-toxic dose. Cytotoxicity was assessed by MTS assay. LPS-stimulated cells were concomitantly treated with 10 μg/mL and 125 μg/mL NaB. Then inflammatory related gene expression levels, TNF-α, IL-1β, and IL-8, were evaluated by qRT-PCR analysis.
RESULTS: all LPS concentrations did not show any cytotoxic effect for 24h. 62.5-500 μg/mL NaB did not display cytotoxicity for 24h. 1000 μg/mL NaB treatment was toxic for HMC3 cells. According to MTS results, 10 μg/mL LPS and 125 μg/mL NaB were selected for qRT-PCR analysis. 10 μg/mL LPS caused upregulation of TNF-α, IL-1β, IL-6 and IL-8 gene expression levels. Concomitant

125 µg/mL NaB treatment attenuated the mRNA levels of these inflammatory cytokines in LPS-stimulated cells.
CONCLUSIONS: our results indicate that NaB suppresses the expression of pro-inflammatory cytokines and exerts anti-inflammatory effects in an *in vitro* model of neuroinflammation.

ASSOCIATION BETWEEN INSULIN RESISTANCE AND SKELETAL MUSCLE MITOCHONDRIAL CONTENT IN INDIVIDUALS WITH RHEUMATOID ARTHRITIS

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OBJECTIVE: Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease associated with increased prevalence of insulin resistance (IR) (Quevedo-Abeledo JC, *et al.* Higher Prevalence and Degree of Insulin Resistance in Patients with Rheumatoid Arthritis Than in Patients with Systemic Lupus Erythematosus. J Rheumatol. 2021;48(3):339-47) and increased cardiovascular disease risk (Avina-Zubieta JA, *et al.* Risk of incident cardiovascular events in patients with rheumatoid arthritis: a meta-analysis of observational studies. Ann Rheum Dis. 2012;71(9):1524-9). Mitochondrial dysfunction has been implicated in the pathogenesis of IR (Montgomery MK, *et al.* Mitochondrial dysfunction and insulin resistance: an update. Endocr Connect. 2015;4(1):R1-R15). The objective of this study was to determine mitochondrial abundance in skeletal muscle and to investigate its association with IR in RA individuals.
METHODS: a prospective, cross-sectional study was performed in RA individuals (n = 24) and healthy controls (n = 20) comparable in age/sex/BMI. Citrate synthase activity (CSA) in the skeletal muscle homogenates obtained from the vastus lateralis and the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) were used as measures of mitochondrial content and IR, respectively. Spearman's correlation was performed to determine the correlation between mitochondrial content with HOMA-IR among RA individuals.
RESULTS: RA subjects demonstrated significantly higher levels of IR [1.75 (1.35, 3.01) *vs*. 1.04 (0.78, 1.72); $p = 0.006$] and lower levels of CSA compared to controls [60 mU/mg (IQR: 45, 80) *vs*. 79 mU/mg (IQR: 65, 97); $p = 0.025$]. However, we did not observe a significant correlation between skeletal muscle CSA and HOMA-IR among individuals with RA (rho = - 0.18, $p = 0.39$).
CONCLUSIONS: we did not find evidence that IR is significantly associated with lower skeletal muscle mitochondrial content in individuals with RA.

AGE-ASSOCIATED DYSREGULATION OF LEUKOCYTE TRAFFICKING DURING ACUTE INFLAMMATION: A POTENTIAL THERAPEUTIC ROLE FOR PEPITEM?

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OBJECTIVE: ageing is associated with abnormal leukocyte trafficking into peripheral tissues, which underpins the development of age-related inflammatory diseases (ARIDs). The immunoregulatory peptide, PEPITEM, is secreted by adiponectin-stimulated B-cells and acts to limit T-cell trafficking, but becomes dysregulated in some inflammatory diseases. Here we investigate the effects of ageing on the ability of the adiponectin-PEPITEM pathway to control of leukocyte trafficking in mice and humans.
METHODS: young (3 mo) and aged (21 mo) mice were subjected to peritonitis in the presence or absence of PEPITEM. Leukocyte subpopulations in the peritoneal lavage fluid were quantified at 48 h using flow cytometry. Peripheral blood lymphocytes (PBL) were isolated from young (23-36 yo) and older (60-75 yo) healthy donors. Expression of adiponectin receptors (AdipoR) on B-cells was assessed using flow cytometry, whilst PBL migration across inflamed endothelial cells was visualized by phase contrast microscopy.
RESULTS: at baseline, aged mice had significantly more lymphocytes and neutrophils but less macrophages in the peritoneum when compared to young. Similarly, significantly more lymphocytes migrated into the inflamed peritoneum in aged mice compared to young mice. Crucially, PEPITEM treatment reverses this age-associated response. Older adults have reduced frequencies of AdipoR1$^+$ circulating B-cells compared to young individuals. Unlike the young cohort, PBL from older adults were unresponsive to adiponectin and failed to limit PBL migration.
CONCLUSIONS: ageing modulates leukocyte trafficking during homeostasis and inflammation, and is associated with PEPITEM pathway dysfunction. PEPITEM treatment can reverse the effects of ageing on leukocyte trafficking, thus offering a novel avenue of investigation to limit onset of ARIDs.

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THE LONG NON-CODING RNA *H19* AS A REGULATOR OF MACROPH-AGEING

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OBJECTIVE: the long non-coding RNA *H19* is downregulated in inflammatory diseases and with advanced age, but its involvement in inflammatory diseases is ill-defined. Macrophages critically contribute to inflammaging *via* increased cytokine release and reactive oxygen species (ROS) production. In parallel, macrophage proliferation, phagocytosis, autophagy, and bactericidal capacity are impaired in the elderly. Thus, we aimed to investigate the influence of *H19* on macrophages.

METHODS: alveolar, peritoneal, and bone-marrow-derived macrophages from wild-type (WT) and *H19* knockout (KO) macrophages were used. Gene expression was either determined by qPCR, flow cytometry, or Western blot. Tumour necrosis factor (TNF) release was measured by ELISA and bioassay, and reporter cells were used to assess NF-kB and AP-1 activity. The phagocytotic capacity was quantified by live-cell microscopy and flow cytometry, and the bactericidal activity was evaluated

by quantification of live intracellular bacteria after *Salmonella typhimurium* infection. Energetic profiles were generated with the Seahorse flux analyzer. Macrophage proliferation was determined by live-cell microscopy and quantification of the proliferation marker Ki67.
RESULTS: *H19* KO led to an inflammatory effect, as indicated by elevated TNF secretion. Overexpression of *H19* in NF-kB/AP-1 reporter cell lines attenuated the activity of these pro-inflammatory transcription factors, thereby confirming that *H19* exerts anti-inflammatory actions. Phagocytosis, bactericidal activity, and autophagosome formation rate were reduced in *H19* KO macrophages. Moreover, mitochondrial ATP production was increased, suggesting that *H19*-deficiency leads to reduced mitophagy and elevated mitochondrial ROS production.
H19 increased the proliferation of bone marrow-derived macrophages *in vitro* and alveolar macrophages *in vivo*. *H19* knockout resulted in enhanced expression of the antiproliferative factor runt-related factor-1 (RUNX1), an established target of the *H19*-derived microRNA miR-675, implicating the signalling pathway *H19*/miR-675/RUNX1 as the underlying molecular mechanism. Reduced alveolar macrophage proliferation was also observed in aged mice. In accordance, macrophages from old mice showed decreased levels of *H19,* paralleled by an up-regulation of *Runx1.*
CONCLUSIONS: our study showed that *H19* depletion skews macrophages towards an ageing phenotype. Thus, *H19* may serve as a potential target in age-related inflammatory diseases.

ELLAGIC AND PUNICIC ACIDS EXTRACTED FROM *PUNICA GRANATA* REDUCE INFLAMMATION IN AN *IN VITRO* MODEL OF EXCITOTOXICITY

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OBJECTIVE: excitoxicity is a neuronal death process induced by the massive release of the excitatory amino acids; it is triggered by glutamate receptor activation and is the main cause underlying epileptic seizures. Preventing neuroinflammation can exert neuroprotective effects and reduce the long term sequelae of brain damage. Several studies demonstrated that Ellagic and Punicic acids (EA, PA) (Pieróg M, *et al.* Effect of Ellagic Acid on Seizure Threshold in Two Acute Seizure Tests in Mice. Molecules. 2021;26(16):4841), found in several fruits including pomegranates, have anti-inflammatory and anti-oxidant effects (Ambrogini P, *et al.* Excitotoxicity, neuroinflammation and oxidant stress as molecular bases of epileptogenesis and epilepsy-derived neurodegeneration: The role of vitamin E. Biochim Biophys Acta Mol Basis Dis. 2019;1865(6):1098-112). The aim of the study was to evaluate whether these polyphenolic compounds were able to reduce neuroinflammation and apoptosis in an in vitro model of kainic acid-induced excitotoxicity.
METHODS: astrocytes were treated with ellagic and punicic acids alone and in combination at different concentrations for up to 24 h, following the excitotoxic stimulus of 50 μM KA. The combination of the 2 extracts was also tested for synergistic effect using as endpoint the expression of Bcl-2 as an apoptotic marker.
RESULTS: Ellagic and Punicic acids increased cell viability and reduced apoptosis pathway, down- regulating BAX and Caspases 3/9 expression and up-regulating Bcl-2 level. Tested compounds reduced expression of pro-inflammatory cytokines, especially IL1-β, IL-6, TNFα and expression of NLRP3. Also extracellular signal-regulated kinase (ERK) pathway is involved in response to extracellular stimuli during neurotoxicity and our compounds reduced ERK and increased the expression of PPARγ, known for its anti-inflammatory effects.
CONCLUSIONS: these preliminary results suggest that the effects of these two natural compounds during excitotoxic response relies on the reduced expression of pro-inflammatory cytokines.

THE EFFECT OF ANTI-INFLAMMATORY DRUGS ON THE NEUTROPHILS EXTRACELLULAR TRAPS FORMATION

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OBJECTIVE: currently, the anti-inflammatory activity of pharmacological drugs is associated primarily with their ability to block the synthesis or release of inflammatory mediators. The effect of these drugs on such functions of inflammatory cells as phagocytosis and netosis, which play an important role in the formation of the inflammatory response, has not been studied. In case of netosis, a network of neutrophilic DNA fibers captures and retains microbial and damaged cells, followed by phagocytosis of the formed structures. With a prolonged inflammatory reaction, the formation of neutrophilic extracellular traps causes secondary tissue alteration. The purpose of this study was to determine the number of neutrophilic extracellular traps under the influence of the classic nonsteroidal anti-inflammatory drug Diclofenac and the local anti-infective therapy drug benzydamine.
METHODS: within the framework of the study, peripheral blood neutrophils from 12 patients with acute inflammatory diseases (abdominal abscess) were used. In order to detect and enumerate neutrophil extracellular traps, fluorescence microscopy using a specific double-stranded DNA dye Syber Green (Evrogen) was used. The investigated pharmacological agent was added to the sterile isolated neutrophils, and incubation was carried out in an atmosphere of 5% CO_2 at 37 °C.
RESULTS: Diclofenac inhibits the opening of neutrophilic extracellular traps when used at a concentration of 3 μg/mL, which optimal for humans, and inhibits the opening of traps by no more than 30%. Under the influence of benzydamine at a concentration of 15 μg/mL, the formation of neutrophilic extracellular traps increases by 20%. Benzydamine at a low concentration (0.15 μg/mL) causes a significant inhibition of the formation of neutrophilic extracellular traps (by 5 times). Such a biphasic effect of benzydamine can be explained by the structure of the active center of the molecule, homologous to the active center of phosphatidylserine, which is a physiological inducer of the formation of neutrophilic extracellular traps.
CONCLUSIONS: the results obtained show that the combination of classical non-steroidal anti-inflammatory drugs with drugs with competitive inhibitors of phosphatidylserine is promising.

MECHANISMS OF POST-COVID INFLAMMATION

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OBJECTIVE: post-COVID syndrome develops in 20% of patients and is characterized by high fatigue, decreased exercise tolerance, muscle and joint pain, the presence of psychoemotional problems and cognitive impairments. We conducted a study of neutrophils extracellular traps (NET) characteristic of inflammation and DNA fibers degradation products (purine nitrogenous bases, PNB), as well as a number of biochemical parameters.
METHODS: the study included 24 patients with severe cognitive disorders and 21 patients without cognitive disorders. The control group consisted of 20 healthy donors without a previous coronavirus infection.
RESULTS: in patients with severe cognitive impairment in PNB in the blood and the concentration of neurotoxic metabolites were increased. In the blood of these patients, the concentrations of quinoline, xanthurenic and quinoline acid were significantly increased by 2-3 times higher than the control values.
The excess of these acids stimulates NMDA receptors and causes the entry of calcium ions into the cell, activation of intracellular proteases and generation of reactive oxygen species, causing cell damage, and in severe cases, the death of neurons.
In the group of patients without severe cognitive disorders, weakness, headache, epigastric pain, dizziness, joint pain were most often observed. The concentration of NET and PNB was higher in these patients than in the control group ($p < 0.05$). In patients, we detected filiform NET. In patients who have had an acute period of the disease in mild form, the concentration of PNB was 23.27 ± 8.9, and in patients with severe form - 35.84 ± 19.25 mg/ml, respectively.
CONCLUSIONS: we consider that there are two mechanisms of post-COVID inflammation. The first is metabolic, leads to the formation of neurotoxic molecules and causes cognitive disorders. The second is associated with accelerated degradation of cells of innate or adaptive immunity, the release of DNA and its degradation to extracellular purine nitrogenous bases in concentrations that cause secondary cell alteration and damage to internal organs.

BARRIOLIDES: NON-ANTIBACTERIAL COMPOUNDS WITH EPITHELIAL BARRIER ENHANCING PROPERTIES AND ANTI-INFLAMMATORY EFFECTS *IN VITRO*

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OBJECTIVE: the macrolide antibiotic azithromycin (Azm) is well-known for its antibacterial properties. On long term treatment, Azm mitigates non-infectious chronic airway diseases (CAD) like COPD but promotes macrolide resistant microorganisms. Our research on Azm led to the development of several 15-membered macrolides lacking antibacterial activity but augmenting epithelial integrity *in vitro*.
METHODS: our compounds, known as "barriolides", have been studied in several *in vitro* systems for their effects on bronchial epithelium. Using air-liquid interface cultures of several lung epithelial cell lines, we have shown that barriolides promote transepithelial electrical resistance and decreased permeability, similar to Azm.
RESULTS: we have observed alterations by barriolides to intracellular lipid metabolism, enhanced expression of cell junction proteins, and altered gene regulation of several ontology groups including water loss and oxidative stress. Although several barriolides have overlapping effects, their gene signature in cells is unique to each. High magnification of the cells indicated an increase in multivesicular- and lamellar bodies, supporting the involvement of lipid metabolism. Anti-inflammatory potential was examined using isolated human macrophages and neutrophils. While there were some similarities to Azm, our lead compound displayed slight differences in its regulation of M1 macrophage polarisation. In neutrophils, both native and stimulated, our lead compound reduced ROS and LL-37 release indicating its effects on tissue-damaging mediators.
CONCLUSIONS: these data give support to the potential for barriolides to enhance respiratory epithelial barrier integrity, possibly via lipid metabolism, and to modulate inflammatory responses.

TARGETING THE LUNG EPITHELIAL BARRIER TO INHIBIT NEUTROPHILIC INFLAMMATION

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OBJECTIVE: respiratory epithelium acts as a first line of defence against external stimuli of biological and material origin. Macrolide antibiotics display disease-modifying effects in addition to their primary antimicrobial activities. Evidence supporting a dual role of macrolides in reducing inflammation and promoting barrier repair led us to develop non-antibiotic compounds with similar qualities while avoiding antimicrobial resistance. We now introduce a new class of compounds, "barriolides", based on an azithromycin (Azm) backbone, a well-tolerated and highly prescribed macrolide antibiotic commonly used in the treatment of exacerbations in patients with chronic airway diseases. Barriolides are targeted to enhance the airway epithelial barrier and inhibit inflammation.
METHODS: using a cigarette-smoke inflammation mouse model, the lead compound dose-dependently reduced neutrophil infiltration and concentrations of key inflammatory cytokines, TNFα and IL-6, after 2 weeks' pre-treatment. In another mouse model involving SO_2 gas exposure, pre-treatment with the set of barriolide compounds reduced permeability of exogenously (tail vein) injected human serum albumin into the bronchoalveolar fluid.
RESULTS: across both *in vivo* models, our compounds display significant anti-inflammatory properties, comparable to those of Azm.
CONCLUSIONS: these data give support to the potential for barriolides to modify diseases involving neutrophilic infiltration and epithelial barrier dysfunction.

ADENOSINE (A METABOKINE) BASED TARGETING OF MACROPHAGES DURING *KLEBSIELLA PNEUMONIAE* B5055-INDUCED ACUTE LUNG INFECTION

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Adenosine is considered as a potent metabokine with potential immunoregulatory function. It is produced during condition causing metabolic stress including hypoxia and inflammation. In current study, we have investigated the effect of 2-chloroadenosine (2-CADO) an analogue of adenosine on the lung innate immune response during acute lung infection induced by *Klebsiella pneumoniae* B5055. Acute lung inflammation was induced by intranasal instillation of *K. pneumoniae* B5055 into mice. Subsequently mice were treated with 2-CADO (10 µg/kg/day/iv) using a treatment schedule. 2-CADO treatment modulated pro-inflammatory function of alveolar macrophages by significantly ($p \leq 0.05$) decreasing their phagocytic activity, nitric oxide (NO) and hydrogen peroxide (H_2O_2) release. 2-CADO also significantly ($p \leq 0.05$) decreased neutrophil infiltration into the lungs. Levels of pro-inflammatory cytokines (IL-1α and TNF-α) were decreased significantly ($p \leq 0.05$) decreased. However, levels of IL-10 were found to be significantly ($p \leq 0.05$) elevated. Thus, adenosine, a metabokine has promising immunomodulatory action during Gram negative bacterial pneumonia.

ON104, A NEXT-GENERATION ANTIBODY TARGETING OXIDIZED MACROPHAGE MIGRATION INHIBITORY FACTOR (oxMIF), IN EXPERIMENTAL MODELS OF GLOMERULONEPHRITIS AND RHEUMATOID ARTHRITIS

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OBJECTIVE: Macrophage Migration Inhibitory Factor (MIF) is a pleiotropic inflammatory cytokine playing an important role in innate and adaptive immunity and emerged as a pivotal regulator in chronic inflammation and autoimmune diseases such as glomerulonephritis and rheumatoid arthritis. Targeting the disease-related isoform oxMIF, which in contrast to the reduced isoform (redMIF) is characterized by a markedly specific expression in inflamed tissues, represents a new and promising treatment option for patients with autoimmune disorders.
METHODS: by antibody engineering, we developed the antibody ON104 which has excellent biophysical properties and is specifically directed against oxMIF. ON104 was tested in two different inflammation models, immune mediated-nephrotoxic nephritis (NTN) in rats and collagen-induced arthritis (CIA) in mice. For the NTN model, proteinuria, haematuria, and histology were used as a read-out. In the CIA model, the severity of the disease was assessed by clinical scoring of paw swelling.
RESULTS: only two intraperitoneal administrations of ON104 significantly reduced the severity of experimental NTN, showing superior anti-inflammatory activities compared to a first generation, clinical anti-oxMIF antibody. In the CIA model, ON104 treatment also substantially improved the clinical symptoms compared to the vehicle-treated control. Since MIF is considered as a primary counter-regulator of glucocorticoids (GCs), combinations of sub-therapeutic doses of dexamethasone and ON104 are currently tested in both models.
CONCLUSIONS: ON104 has the potential to become a well-tolerated non-steroidal anti- Inflammatory drug for patients with chronic inflammatory diseases. Due to MIF's hallmark to override anti-inflammatory effects of GCs, synergistic effects of ON104 and steroidal drugs are likely why ON104 may represent a new treatment option for patients having significant GCs side effects or are resistant to GCs.

MELATONIN ADMINISTRATION AFTER IRISIN SILENCING AFFECTS ADIPOSE TISSUE INFLAMMATORY AND METABOLIC PATHWAYS

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OBJECTIVE: physical activity has been exploited as an effective mean for preventing and treating several pathological conditions such as obesity and metabolic disorders (Albrecht E, *et al.* Irisin: Still chasing shadows. Mol Metab. 2020;34:124-35). Irisin, a myokine released from muscle influences metabolic pathways in several districts, such as the adipose tissue (Li H, *et al.* The Effect of Irisin as a Metabolic Regulator and Its Therapeutic Potential for Obesity. Int J Endocrinol. 2021;2021:6572342), where promotes thermogenesis, lipolysis and mitigate inflammatory processes. It has been recently found that the expression of irisin is regulated by melatonin, a neurohormone largely known for its anti- inflammatory and antioxidant role (de Farias TDSM, *et al*. Melatonin Supplementation Decreases Hypertrophic Obesity and Inflammation Induced by High-Fat Diet in Mice. Front Endocrinol. 2019;10:750.). Therefore, the aim of this study was to determine whether melatonin effects could be related to irisin activity in the adipose tissue, especially when it was silenced through a silencing RNA (siRNA).
METHODS: animals were trained for two consecutive weeks, and randomized to receive: i) melatonin; ii) FNDC5 silencing RNA; iii) melatonin plus the FDNC5 siRNA; or iv) saline solution. At the end of the experimental procedure fat samples were collected to evaluate mRNA expression of: adipokines (Adiponectin and Visfatin), targets of white adipose tissue (FASN and FABP4), and targets related to adipocytes metabolic and inflammatory profile (FNDC5, PGC1α, SIRT1, PPARγ, IL-6 and TNF-α).
RESULTS: irisin silencing increased the expression of pro-inflammatory adipokines, and also enhanced the expression of genes typical of white adipocytes. In contrast, melatonin administration significantly reduced the levels of the above targets, suggesting an attenuation of adipose tissue inflammation. The increased expression of PPARγ suggests that melatonin may promote: i) the reduction of lipid concentration, ii) the expression of PGC1α, and, consequently, of FNDC5. Melatonin supplementation after irisin silencing was able to provide a profile similar but not identical to that resulting from the administration of melatonin.

CONCLUSIONS: the overall findings, confirming the anti-inflammatory activity of irisin in adipocytes, also suggest that melatonin is likely to enhance the positive effects promoted by irisin by mechanisms that are not yet fully elucidated but that might be related to the different melatonin receptors.

THE INVERTEBRATE *Ciona robusta* AS A MODEL FOR STUDYING INFLAMMATORY MECHANISMS

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OBJECTIVE: *Ciona robusta* is a cosmopolitan marine invertebrate (Tunicata, Chordata) with a short life span consisting of embryonic, larval, juvenile, and adult phases. Due to its phylogenetic position, it is a model for comparative studies on the inflammatory response of the innate immune system. Different immune genes have been identified in *Ciona* genome, such as pattern recognition receptors, cytokines, complement system and transcription factors. Here, we report the differential activation of the innate immune system upon exposure to inflammatory agents.
METHODS: crosstalks of the innate immune system with the external environment begins in 5 days post fertilization *Ciona* juveniles at the onset of seawater filter feeding. This juvenile stage is thus suitable for investigating the effects of microbial stimuli (LPS, Pam2CSK4, Zymosan) on immune response. We used different exposure concentrations and times to evaluate the inflammatory response through the expression analysis of selected immune genes by RT-qPCR.
RESULTS: at first, an *in silico* search of immune genes through an homology domain analysis, using specific databases and bioinformatic tools, was performed. This approach allowed to identify a wide number of immune related genes shared with human to be studied at the transcriptional and biochemical levels. Our main findings demonstrate that microbial stimuli differentially affect immune pathways activation, especially concerning the expression of transcription factors (*i.e. NFkB*, *IRF-like* and *NFat5*) and cytokines (*IL-17s*) coding genes.
CONCLUSIONS: we have developed a *C. robusta* inflammatory model by describing the immune response to different microbial stimuli upon a relatively short time exposure. We are currently developing HTP protocols for rapidly investigating the effect of bioactive molecules on the innate immune response of this non-mammalian inflammatory model. *C. robusta* represents a unique opportunity for using a whole organism that display conserved molecular mechanisms with mammals in translational research.

ELUCIDATING THE ROLE OF ENDOTHELIAL GALECTINS IN LEUKOCYTE TRAFFICKING

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OBJECTIVE: Glycan-binding proteins (GBPs) are key facilitators of leukocyte trafficking from the bloodstream to tissue during the initial stages of the inflammatory response. Galectins, a class of GBPs, have been shown to play critical roles at key stages of inflammatory responses and have been linked to the pathogenesis of inflammatory diseases. Despite the known roles for galectins in modulating leukocyte trafficking events, the specific influence of endothelial-galectin interactions remains elusive.
METHODS: we treated endothelial cells from various vascular sources with TNFα, IFNγ or combined treatment and analysed changes in transcription of LGALS1, LGALS3, LGALS8 and LGALS9 by qPCR. Additionally, we used galectin-peroxidase fusion proteins to label and identify potential galectin interactors expressed in live human umbilical vein endothelial cells (HUVEC) in response to cytokine treatment.
RESULTS: we observed that galectin-3 and -9 are transcriptionally modulated in HUVEC following TNFα and IFNγ treatment, to a much greater extent than treatment with individual constituents alone. We have also found that endogenous endothelial galectin expression is modulated in response to shear stress, highlighting the importance of studying endothelial-galectin functions in near-physiological shear stress environments. Thus, we have identified pro-inflammatory mediators and shear stress as regulators of endogenous endothelial galectin expression, and have begun to analyse the functional consequences of specific endothelial-galectin interactions on leukocyte trafficking events using flow-based adhesion assays to mimic micro- and macro- vascular systems *in vitro*.
CONCLUSIONS: we predict that galectin-endothelial interactions are critical for leukocyte trafficking and would make an appropriate target for therapeutic intervention.

CARBONIC ANHYDRASE IV SELECTIVE INHIBITORS COUNTERACT THE DEVELOPMENT OF COLITIS-ASSOCIATED VISCERAL PAIN IN RATS

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OBJECTIVE: pain management represents a clinical problem in patients affected by inflammatory bowel diseases (IBDs) since effective and safe therapies lack. Of note, 20-50% of patients manifest abdominal pain also in the remission phase of colitis, because of sensory pathways sensitization during inflammation. Recent evidence highlighted the antihyperalgesic efficacy of carbonic anhydrase (CA) inhibitors in both inflammatory and neuropathic pain models. The aim of this work was to evaluate the effect of inhibiting the isoform CA IV, particularly expressed in the gut, on colitis-associated visceral pain in rats.

METHODS: colitis was induced by DNBS intra-colonic instillation. The selective CA IV inhibitors, AB-118 and NIK-67, were daily administered for 14 days after DNBS injection. Visceral sensitivity was assessed by measuring animals' abdominal responses to colorectal distension.
RESULTS: the repeated treatment with AB-118 and NIK-67 effectively counteracted the development of visceral pain induced by DNBS. In addition to pain relief, AB-118 showed a protective effect on colon damage. By contrast, the antihyperalgesic activity of NIK-67 resulted independent from tissue healing, suggesting a specific modulation of visceral sensitivity. Either the enzymatic activity and the expression of CA IV (in colon epithelial and neuromuscular layers) resulted significantly increased after DNBS injection. NIK-67 normalized CA IV activity in DNBS animals, while AB-118 was partially effective. Both the compounds did not influence CA IV expression through the colon.
CONCLUSIONS: although further investigations are needed to study the underlying mechanisms, CA IV inhibitors are promising candidates in the search of therapies for relieving visceral pain in IBDs.

NEUTROPHIL FORMYL PEPTIDE RECEPTORS (FPRs) AS NOVEL PHARMACOLOGICAL TARGETS FOR RHEUMATOID ARTHRITIS: PRECLINICAL ASSESSMENT

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OBJECTIVE: neutrophil formyl peptide receptors (FPR1, FPR2 and FPR3) are members of the G-protein-coupled receptor family. Although not completely understood, their role in the inflammatory response modulation is emerging as a new therapeutic target. FPR agonists could offer a valuable approach mainly to chronic inflammatory diseases, such as rheumatoid arthritis.
METHODS: the action of the novel pyridinone compound 2a, with good activity for FPR2 (EC_{50} = 0.12 μM), was evaluated *in vitro* in an inflammatory model induced by IL-1β (10 ng/mL) on a rat primary chondrocyte's culture. Moreover, 2a was tested *in vivo* in a rat model of rheumatoid arthritis induced by complete Freund's adjuvant (CFA) intra-articular injection. *Ex-vivo* evaluations on the spinal cord were also performed.
RESULTS: on chondrocytes, the treatment with 2a reduced the oxidative stress (assessed by both ROS and catalase activity assays), as well as the expression of pro-inflammatory genes, and improved the collagen fibres organization (assessed by PAS-staining), compared to IL-1β-induced damage. CFA-injected animals daily treated for two weeks with 2a (10 mg/kg, *per os*) showed a significant increase of pain threshold and reduction of postural unbalance in comparison to those treated with vehicle, both 7 and 14 days after damage induction. The histological evaluation of the joint highlighted a decrease of inflammatory infiltrate and partial preservation of joint space following the treatment. Furthermore, 2a administration counteracted spinal cord astrocyte activation (assessed by GFAP fluorescence intensity, number of $GFAP^+$-cells, and morphological parameters) and modulated the expression of inflammatory and pain-related genes (CCL2, EAAT1, EAAT2, VEGF-A, GFAP, S100β).
CONCLUSIONS: this new FPR agonist represents a valid candidate for the treatment of rheumatoid arthritis.

METABOLITE SIGNATURES CAN DEFINE DISEASE, RESPONSE TO GLUCOCORTICOID TREATMENT AND FATIGUE IN PATIENTS WITH PMR

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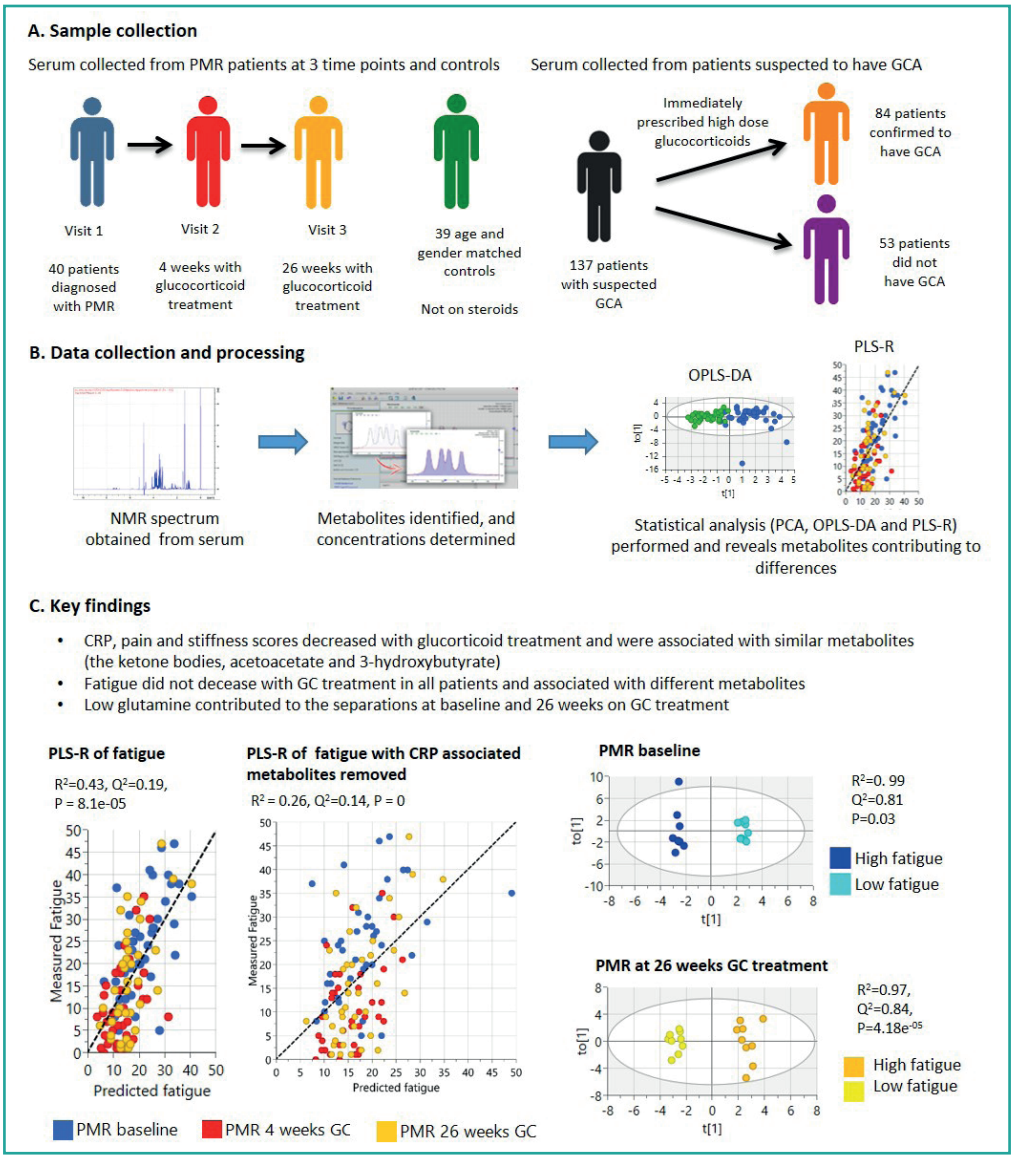
OBJECTIVE: in polymyalgia rheumatica (PMR) and giant cell arteritis (GCA), glucocorticoids relieve pain and stiffness, but fatigue persists in some patients. Our aim was to explore the relationship of these three symptoms to metabolite signatures in peripheral blood.
METHODS: nuclear magnetic resonance spectroscopy was performed on serum from 40 patients with untreated PMR, 84 with new-onset confirmed GCA, and 53 with suspected GCA who later received other diagnoses, and 39 age-matched controls. Further samples from PMR patients were taken 1 and 6 months into glucocorticoid therapy to explore relationship of metabolites to persistent fatigue. 100 metabolites were identified using Chenomx and statistical analysis performed in SIMCA-P to examine the relationship between metabolic profiles and disease or symptoms.
RESULTS: the metabolite signature of patients with PMR and GCA differed from that of age-matched non-inflammatory controls (R^2 > 0.7). There was a smaller separation between patients with clinically-confirmed GCA and those with suspected GCA who later received other diagnoses (R^2 = 0.135). In PMR, metabolite signatures were further altered with glucocorticoid treatment (R^2 = 0.42), but did not return to that seen in controls. Metabolites correlated with CRP, pain, stiffness and fatigue ($R^2 \geq 0.39$). CRP, pain, and stiffness declined with treatment and were associated with 3-hydroxybutyrate and acetoacetate, but fatigue did not. Metabolites differentiated patients with high and low fatigue both before and after treatment ($R^2 > 0.9$). Low serum glutamine was predictive of high fatigue at both time points (0.79 fold change).
CONCLUSION: PMR and GCA alter the metabolite signature. In PMR, this is further altered by glucocorticoid therapy. Treatment-induced metabolite changes were linked to measures of inflammation (CRP, pain and stiffness), but not to fatigue. Furthermore, metabolite signatures distinguished patients with high or low fatigue.



AMBRA1 AND LC3B STIMULATION BY ELLAGIC AND PUNICIC ACIDS PROMOTE MITOPHAGY AND AUTOPHAGY FOLLOWING KAINIC ACID INDUCED EXCITOTOXICITY IN NEURONAL CELLS

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BACKGROUND: epilepsy is the second most common neurological disorder mainly due to excitotoxicity, a complex process triggered by an excessive release of glutamate that over-activates its receptors, resulting in increased levels of intracellular calcium which promotes the production of ROS, releases caspase cofactors and causes ER stress-mediated apoptosis. Meantime, excessive accumulation of ROS may lead to the subsequent reduction of autophagy and AMBRA1-induced mitophagy in an attempt to scavenge damaged cellular components (Fassio A, *et al.* Emerging Role of the Autophagy/Lysosomal Degradative Pathway in Neurodevelopmental Disorders with Epilepsy. Front Cell Neurosci. 2020;14:39).
Since excitotoxicity is still a major problem in neuroinflammatory conditions several approaches, including the nutraceuticals have been tested in recent years (Cao J, *et al.* Hyperoside alleviates epilepsy-induced neuronal damage by enhancing antioxidant levels and reducing autophagy. J Ethnopharmacol. 2020;257:112884). The hypothesis here tested is that ellagic and punicic acids, natural polyphenolic compounds derived from pomegranate, might effectively reduce excitotoxic stress in neurons stimulated with a potent activator of glutamate receptors, namely kainic acid (KA).
METHODS: the neuroblastoma cell line SHSY-5Y was differentiated in neurons under appropriate culturing conditions. Cells were treated with ellagic and punicic acids, alone or in combination at the concentration of 1 μM following KA (50 μM) challenge for 24 h to induce an in vitro model of epilepsy.

RESULTS: the obtained results showed that the compounds were able to increase cell viability, reduce ROS production and restore cell morphology compared to KA stimulated cells. Moreover, ellagic and punicic acids reduced oxidative stress, decreasing Keap1 and increasing Nrf2 expression, and increased neural differentiation and synapse development, decreasing cMyc, phospho-b-Catenin and Cyclin D1 expression. Furthermore, polyphenolic compounds caused a significant reduction of mTor, p-Akt, caspase 3/9, whereas promoted a significant increase in the expression of Ambra1, beclin-1 and LC3B.
CONCLUSIONS: these preliminary data suggest that ellagic and punicic acids could reduce excitotoxicity through activation of autophagy and inhibition of oxidative stress, providing evidence on the possible use of these natural polyphenolic compounds as a new therapeutic approach for neuroinflammatory conditions.

THE MECHANISMS INVOLVED IN DIFFERENTIAL RESPONSES AND OUTCOMES OF IDENTICAL MICE TO SEPSIS

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OBJECTIVE: sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. There is estimate an annual occurrence of 31.5 million cases of sepsis causing potentially 5.3 million deaths. New therapies, such as immunotherapies, have been tested in sepsis; but with no success yet. Thus, studies using advanced methods may contribute to the identification of new therapeutic targets in sepsis. By inducing cecal ligation and puncture sepsis model (CLP) in C57/Bl6 mice genetically equal and having the same grown-up environment, these animals have different response to sepsis in which half of the mice survive and the other half die from sepsis. That way, our study aims to identify new therapeutic targets by analyzing the profile of the different leukocyte subtypes comparing surviving and non-surviving septic animals.
METHODS: we induced sepsis in C57/Bl6 male mice using Cecal ligation and puncture (CLP), the gold standard model of sepsis. Moderate and severe intensities of CLP sepsis were performed using a 21- or 18-gauge needle, respectively. Of note, induction of severe CLP was followed by antibiotic treatment (ertapenem sodic). In both cases, CLP promoted mortality of 50%. Animal Research Ethical Committee number: 151/2019.
RESULTS: after 6 hours of CLP induction, surviving and non-surviving mice displayed the same serum levels of cytokines (IL-6, IL-10, CXCL1, CXCL2, and CCL2). However, at 12 h, 24 h, or 48 h, animals that survived from sepsis presented reduced levels of these cytokines, while in non-surviving mice these levels remained higher. Non-surviving mice also showed an increase of plasma concentration of liver, kidney, and heart lesion biomarkers and bacteremia. Likewise, non-surviving mice showed increased concentrations of chemokines and cytokines in the lungs, kidneys, heart, liver, and also in the primary infection focus (peritoneal cavity) in comparison with surviving animals at 24 h after CLP. By evaluating the activation of neutrophils, which are key cells promoting infection control in the CLP model, we observed that both surviving and non-surviving animals displayed decreased CXCR2 expression and an increased CD11b expression at 6h on blood neutrophils. However, after 24 h and 48 h of CLP induction, once neutrophils of surviving mice reestablish CXCR2 and CD11b expression levels similar to the control mice, the neutrophils of non-surviving mice remained with an internalized CXCR2 and high CD11b expression.
CONCLUSIONS: we observed that identical mice responded to the experimental sepsis differently. Notably, non-surviving animals had a persistently higher level of inflammatory cytokines in plasma and organs, and persistent activation of neutrophils. Currently, we are evaluating what conditions might be responsible for these different outcomes in identical mice.

BIOPSYCHOSOCIAL DETERMINANTS OF DISEASE EXPERIENCE IN RHEUMATOID ARTHRITIS

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AIM: to introduce and discuss the uses of the Biopsychosocial Determinants of Disease Experience in Rheumatoid Arthritis (BDRA), a middle range theory, in patient care and research.
BACKGROUND/RATIONALE: Rheumatoid arthritis (RA) is a complicated autoimmune disease that causes pain, fatigue, joint deformity, disability, and risk for serious sequela in approximately 1% of the global population. RA treatment focuses on sustained remission, but evidence suggests only 5-45% achieve this regardless of pharmacological therapy. Repeated RA exacerbations result in joint and bone damage and deformity, financial burden, disability, and an increased risk of death. The Biopsychosocial Model of Disease Experience in Rheumatoid Arthritis (BDRA) is an evidence-based model that considers individual biological, psychological, and sociological determinants of disease activity and subsequent experience. Engel's Biopsychosocial Model of Health (BMH) and the Revised Symptom Management Conceptual Model (RSMCM) are the foundation of a blended theory approach used to develop the BDRA. The broad approach of the BMH illustrates overlapping relationships between biological (gender, age, medications, body mass index, nutrigenomics, microbiome, diet, physical activity), psychological (perceived stress, sleep, tobacco product use, knowledge, self-efficacy), and social (disability, social inclusion, financial resources, culture) constructs that are essential in understanding interactions between immune, neurological, endocrine, and gastrointestinal function. The RSMCM construct of symptoms experience was altered to consider disease experience in RA. This approach allowed for the individualized identification of antecedents of inflammation specific to RA. Concept derivation, synthesis, reformulation, and analysis per Walker and Avant were used for model development. Potential biopsychosocial determinants were explored using peer-reviewed publications.
CONCLUSIONS: inflammation, the driving force behind RA disease activity, is impacted by biopsychosocial factors individual to each person diagnosed. These determinants overlap, interact with one another, and impact inflammation via biological, psychoneuroimmunological, and gut-brain-microbiome pathways, and, ultimately, affect disease experience of individuals with RA. Understanding biopsychosocial determinants of RA disease activity and experience is a critical step to inform future research and subsequent treatment of this complicated disease in efforts to achieve sustained remission.

DIET QUALITY AND DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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OBJECTIVE: associations between diet quality and disease activity in adults with rheumatoid arthritis (RA) were examined in this study. Diet quality and disease activity were also compared to perceived stress.
METHODS: fifty adults with RA were recruited from the community for this cross-sectional study. Dietary intake (four weeks) was measured with the Arizona Food Frequency Questionnaire and the Healthy Eating Index – 2015 was used to calculate diet quality scores. The Perceived Stress Scale was used to measure perceived stress. Disease activity was measured with high-sensitivity C-reactive protein and erythrocyte sedimentation rate levels, the Disease Activity Score Including 28 Joints-ESR, and the Health Assessment Questionnaire-Disability Index and Pain Scale.
RESULTS: participant diet quality scores (56; *SD* ± 12) were lower than the national mean (59). Higher diet quality was associated with age (p = .015) and gender (p = .003). Dietary impact on disease activity was reported by 44% of the participants, who were also significantly more likely to report dietary changes (p < .0001). Individuals with higher educational (*e.g.* at least some college) were more likely to report this belief (B = -1.535, p = .023). Significantly higher pain (B = -.396, p = .022) and ESR scores (p = .019) were associated with lower diet quality. Higher HAQ-DI scores were reported by female participants (B = .570, p = .001). HAQ-DI and pain scores were significantly associated with perceived stress (B = .445, p =.001 and B = .289, p = .042, respectively). Eight percent of participants reported the sue of medical cannabis.
CONCLUSIONS: lower diet quality may be associated with more pain and inflammation in rheumatoid arthritis, and higher disability and disease activity may be associated with perceived stress.

THE ROLE OF MONOMERIC C-reactive PROTEIN IN THE ASSESSMENT OF RESIDUAL INFLAMMATORY CARDIOVASCULAR RISK IN PATIENTS WITH SUBCLINICAL CAROTID ATHEROSCLEROSIS

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OBJECTIVE: the aim was to study the role of the monomeric form of C-reactive protein (mCRP) in the assessment of residual inflammatory risk in patients with subclinical carotid atherosclerosis.
METHODS: the study comprised 80 patients of both sexes 53.1±5.8 years old with moderate cardiovascular SCORE risk, the LDL-C level of 2.7-4.8 mmol/L and subclinical hemodynamically insignificant (< 50% stenosis) carotid atherosclerosis (CA). All patients were prescribed atorvastatin therapy to achieve target LDL-C level < 2.6 mmol/L. At the end of the 7 years of follow-up, ultrasonography of the carotid arteries was performed and the level of hsCRP and mCRP measured.
RESULTS: LDL-C level < 2.6 mmol/L was achieved in all patients. CA progressed in 45 (56%) patients. The mCRP level was significantly higher in patients with CA progression than in patients without CA progression (8.8 ± 7.54 µg/L *vs*. 5.96 ± 8.35 µg/L, p < 0.05). The difference in the hsCRP level was not significant (2.23 ± 2.63 mg/L in patients with CA progression vs. 1.46 ± 1.27 mg/L in patients without CA progression, p > 0.05). A significant correlation of the mCRP level with the increase in the mean number of atherosclerotic plaques per patient and the total plaque height was found in both groups. In patients with the mCRP level below the median value 5.2 (3.35; 7.15) µg/L compared to patients with the mCRP level above the median value the baseline mean number of atherosclerotic plaques per patient was 2.54 ± 1.35 vs. 2.17 ± 1.22, the total plaque height was 5.85 ± 3.75 mm *vs*. 6.95 ± 4.15 mm. After 7 years of follow-up, the mean number of atherosclerotic plaques per patient was 2.90 ± 1.41 vs. 3.37 ± 1.51, the total plaque height was 6.95 ± 4.15 mm vs. 8.18 ± 5.17 mm (p < 0.05). In patients with mCRP level above the median value the increase in the mean number of atherosclerotic plaques per patient and total plaque height was significantly higher than in patients with mCRP level below median value (3.9 and 2.7 times, respectively).
CONCLUSIONS: The mCRP plasma level can be used in assessment of residual inflammatory cardiovascular risk.

This work was supported by the Russian Science Foundation grant #22-25-00054.

ASSOCIATION OF HDL PARTICLE SUBFRACTIONS AND PSORIASIS: RESULTS FROM THE BRAZILIAN LONGITUDINAL STUDY OF ADULTS HEALTH (ELSA-Brasil)

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OBJECTIVE: psoriasis is characterized as an inflammatory chronic skin disease. Inflammatory syndromes can influence the high-density lipoprotein (HDL) function and impair the reverse cholesterol transport, affecting the cardiovascular health. Little is known about the association of psoriasis with an abnormal lipoprotein particle profile. We aimed to examine the association between psoriasis and HDL particle (HDL-P) subfractions in a Brazilian population.
METHODS: this cross-sectional study analyzed data from the ELSA-Brasil cohort, collected in 2008-2010. Participants from the Investigation Center of São Paulo with available data of lipid profile measured by nuclear magnetic resonance (NMR) were included. The status of psoriasis was self-reported (yes/no). HDL-P subfractions were analyzed by NMR and defined as small, intermediate, and large based on the mean particle size. We used boxcox transformation on intermediate and large HDL-P variables. Univariate and multivariate linear regression models were done.

The level of significance adopted was $p < 0.05$ and confidence interval of 95% (CI95%).
RESULTS: 4,226 middle-aged adults (50.5 ± 8.6 years old) participated in this study. Most of them were women (54.5%) and 1.4% had psoriasis. Univariate analysis showed no statistically significant associations between psoriasis and small (β = 0.27, CI95% = - 0.54; 1.07, p = 0.517), intermediate (β = - 0.05, CI95% = - 0.34; 0.24, p = 0.752) and large (β = - 0.01, CI95% = - 0.27; 0.25, p = 0.921) HDL-P. After adjustment for sociodemographic variables, comorbidities, lifestyle factors, and cholesterol-related measures, psoriasis remained not associated to the small (β = 0.49, CI95% = - 0.25; 1.23, p = 0.197), intermediate (β = 0.001, CI95% = -0.25; 0.26, p = 0.993), and large (β = 0.01, CI95% = - 0.17; 0.16, p = 0.945) HDL-P subfractions.
CONCLUSIONS: in this large cohort from a Brazilian population, the participants with psoriasis presented a standard HDL profile, showing no association with large, intermediate or small HDL-P subfractions.

ASSOCIATION BETWEEN PSORIASIS AND THYROID DYSFUNCTION: A QUANTILE REGRESSION APPROACH

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OBJECTIVE: psoriasis is an autoimmune inflammatory chronic disease influenced by genetics and environmental triggers. Studies have shown that patients with psoriasis have a higher risk of having thyroid diseases. However, the association with subclinical conditions are still controversial. This study aimed to investigate whether psoriasis is associated with thyroid dysfunction in Brazilian middle-aged and older adults.
METHODS: this is a cross-sectional study with data from the Brazilian Longitudinal Study of Adults Health (ELSA-Brasil), collected during the third visit in 2017-2019. We included all participants with available data, except those using drugs that altered thyroid function. The diagnosis of psoriasis was self-reported and the thyroid function was assessed through hormone tests: thyroid stimulating hormone (TSH), free thyroxine (FT4), and thyroid peroxidase antibodies (TPO-Ab). We performed quantile regressions to identify if psoriasis was associated with different percentiles (10%, 25%, 50%, 75%, and 90%) of TSH, FT4 and TPOAb. Crude and adjusted analyses (sex, age, race, education, smoking, alcohol intake, diabetes, dyslipidemia, BMI, hypertension) were done. The level of significance adopted was $p<0.05$ and confidence interval of 95% (CI95%).
RESULTS: a total of 11,522 participants (59.5 ± 8.8 years old; 55.5% women) were analyzed. The prevalence of psoriasis was 3.0% (n = 343). Crude analysis showed a significant association between psoriasis and the percentile 10 of TSH (β = 0.17, CI95%: 0.04; 0.30; p=0.009), which has kept the significance in the adjusted analyses (β = 0.14, CI95%: 0.01; 0.27; p = 0.043). There was a tendency of association on crude analysis between psoriasis and the percentile 75 of FT4 (β = 0.03, CI95%: - 0.003; 0.06; p = 0.071). After the adjustment, the association was statistically significant (β = 0.03, CI95%: 0.001; 0.07; p = 0.043). There was no association between the percentiles of TPO-Ab and psoriasis ($p > 0.05$).
CONCLUSIONS: our results suggests that psoriasis is associated with lower percentiles of TSH and higher percentiles of FT4. Although, it was not found association between psoriasis and the percentiles of TPO-Ab.

ENHANCED NAMPT-Mediated NAD SALVAGE PATHWAY CONTRIBUTES TO PSORIASIS PATHOGENESIS BY AMPLIFYING EPITHELIAL AUTO-INFLAMMATORY CIRCUITS: POSSIBLE IMPLICATIONS IN THE CO-MORBID CONDITION OF OBESITY

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OBJECTIVE: psoriasis is a chronic inflammatory skin disorder characterized by epidermal alterations and infiltrating immune cells releasing pro-inflammatory cytokines with pathogenic action on resident skin cells. Dysregulated cross-talk between immune cells and epithelial compartments is responsible for the onset and amplification of pathogenic auto-inflammatory circuits occurring in psoriasis (Albanesi C, *et al.* The Interplay Between Keratinocytes and Immune Cells in the Pathogenesis of Psoriasis. Front Immunol. 2018;9:1549). NAMPT-mediated NAD salvage pathway has been recently described as an immunometabolic route having inflammatory function in several disorders, including arthritis and inflammatory bowel diseases (Colombo G, *et al.* Neutralization of extracellular NAMPT (nicotinamide phosphoribosyltransferase) ameliorates experimental murine colitis. J Mol Med. 2020;98(4):595-612). NAMPT has a dual entity: an intracellular form, implicated in NAD biosynthesis, and an extracellular form, with pro-inflammatory proprieties (Galli U, *et al.* Recent Advances in NAMPT Inhibitors: A Novel Immunotherapic Strategy. Front Pharmacol. 2020;11:656).
To date, the role of NAD salvage pathway has not been explored in the skin of patients affected by psoriasis.
METHODS: twenty-five patients with mild-to-severe chronic plaque psoriasis and 10 healthy volunteers were included in this study. Blood and biopsies were taken from skin plaques in both LS and nonlesional (NLS) areas, all from the same psoriatic patients, and from healthy group. Among psoriasis patients, n = 6 received subcutaneous injections of secukinumab. Biopsies were taken from skin plaques at sites overlapping LS before treatment (week 0) and after 8-week treatment and at NLS. These skin biopsies were subjected to Real Time (RT)-PCR and immunohistochemistry analysis to evaluate NAMPT expression. Human keratinocytes, dermal fibroblasts and HDMEC were established from skin of healthy individuals (n = 6 strains), stimulated with inflammatory cytokines, and in vitro analysed in terms of proliferation, migration and expression of immune-related mediators.
RESULTS: NAD content is enhanced in lesional skin of psoriatic patients and is associated to high NAMPT transcriptional levels. The latter are drastically reduced in psoriatic skin following treatment with the anti-IL-17A biologics secukinumab. Serum of psoriasis patients also show enhanced levels of NAMPT, which correlate with severity disease.
Intracellular NAMPT, strongly induced by Th1/Th17-cytokines, acts on keratinocytes by inducing hyper-proliferation and impairing their terminal differentiation. Furthermore, NAMPT-mediated NAD^+ boosting synergizes with psoriasis-related cytokines in the upregulation of inflammatory chemokines important for neutrophil and Th1/Th17 cell recruitment. In addition, extracellular

NAMPT, abundantly released by keratinocytes and dermal fibroblasts, acts in a paracrine manner on endothelial cells by inducing their proliferation and migration, as well as the expression of ICAM-1 membrane molecule and chemokines important for leukocyte recruitment into inflamed skin.
CONCLUSIONS: NAMPT-mediated NAD salvage pathway contributes to psoriasis pathogenic processes by amplifying epithelial auto-inflammatory responses in psoriasis.
Different comorbid conditions co-exist in psoriatic patients. Among these, obesity leads to a higher risk of developing psoriasis with a poorer long-term clinical outcome, reduces the efficacy of conventional psoriasis therapies, and is associated with adverse drug reactions. Ongoing investigations are aimed at elucidating the role of the extracellular NAMPT in the pathogenic networks underlying the co-morbid condition of obesity associated to psoriasis.

A VEGETAL MIXTURE COMPOSED BY *ZINGIBER OFFICINALE, ECHINACEA PURPUREA* AND *CENTELLA ASIATICA* AGAINST NEUROINFLAMMATION EVOKED BY LPS IN MICE

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OBJECTIVES: a large body of experimental evidence suggests that neuroinflammation is a key pathological event triggering and perpetuating the neurorestorative process associated with many diseases affecting the nervous system. Although the cause that drives the progression of each of these neurodegenerative diseases remains elusive, it has been well recognized that these devastating diseases are multifactorial and involve many pathogenic mechanisms reason why current pharmacological treatments are unsatisfactory. In this context, natural products can offer the multiple approach needed to treat the multifactoriality of neuroinflammation. Aim of this study was to evaluate the efficacy of a vegetal mixture composed by *Zingiber officinale*, *Echinacea purpurea* and *Centella asiatica* in a mouse model of neuroinflammation induced by LPS injection.
METHODS: mice were intraperitoneally injected with LPS 1 mg/kg for four alternate days to induce systemic inflammation. Concurrently a vegetal mixture composed by *Zingiber officinale* (150 mg/kg), *Echinacea purpurea* (20 mg/kg) and *Centella asiatica* (200 mg/kg) was daily *per os* administered from day 1until the end of the experiment. Starting from day 9, behavioural measurements were performed to evaluate the effect of the treatment on cognitive impairments, allodynia, motor alterations, anhedonia and depressive-like behaviour evoked by LPS. Histologically, glial analysis of the spinal cord was also conducted.
RESULTS: repeated treatment with the vegetal mixture was able to completely counteract thermal and mechanical allodynia as reported by the Cold plate and von Frey tests, respectively, and to reduce the motor impairments as demonstrated by the Rota rod test. Moreover, the mixture was capable to neutralize the memory loss in the Passive avoidance test, to reduce depressive-like behavior in the Porsolt test and the obsessive-compulsive disorder in the Marble test while no efficacy was shown in decreasing anhedonia as demonstrated by the Sucrose preference test. Finally, LPS stimulation caused a significant increase of central complement proteins that was significantly recovered in animals treated with vegetal mixture.
CONCLUSIONS: the vegetal mixture composed by *Zingiber officinale*, *Echinacea purpurea* and *Centella Asiatica* thwarted the plethora of symptoms evoked by LPS being a candidate for future investigations in the context of neuroinflammation.

N-Palmitoylethanolamine COUNTERACTS NEUROINFLAMMATION DRIVEN BY MORPHINE IN A PRECLINICAL MODEL OF PERSISTENT PAIN

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OBJECTIVE: the clinical application of opioids for persistent pain is limited by the development of tolerance to the analgesic effect and by the misuse due to the facilitation on prescription that frequently leads to several adverse effect. To improve these aspects, novel strategies for the maintenance of low-dosed opioid effectiveness are required. *N*-Palmitoylethanolamine (PEA) is an endogenous compound very well tolerated in humans and endowed with anti-inflammatory properties. In the present study, we evaluated the effect of preemptive and continuative treatments with ultramicronized palmitoylethanolamine (PEA) in modulating morphine analgesia and tolerance in a rat model of neuropathic pain induced by the sciatic nerve ligation (CCI).
METHODS: Sprague-Dawley rats underwent to CCI were used. The animal's pain threshold was daily measured before and 30 min after morphine injection using mechanical noxious and non-noxious stimuli (Paw pressure and Von Frey tests, respectively) while the spontaneous pain was evaluated by the Incapacitance test.
RESULTS: preemptive and continuative PEA treatment (30 mg kg^{-1}, daily, *p.o.*) delayed the onset of morphine (10 mg kg^{-1}, daily, s.c.) tolerance and enhanced the opioid analgesia. The capacity of PEA to potentiate the antinociceptive properties of morphine suggests the possibility to integrate the opioid treatment with PEA for a stable, long-lasting analgesia. To the purpose, morphine dose needed to be increased from 5 mg kg^{-1} (day 1) up to 35 mg kg^{-1} of day 23. A similarly stable analgesic effect was reached using preemptive PEA (30 mg kg^{-1}, daily) joined to a combinatorial acute treatment with morphine (5-7 mg kg^{-1}) and PEA (30 – 60 mg kg^{-1}). Representatively, on day 23, analgesia induced by 35 mg kg^{-1} morphine was reached by the association 7 mg kg^{-1} morphine/60 mg kg^{-1} PEA. Behavioural effects of PEA on opioid chronic treatment matched with a decreased of glial cells activation in the dorsal horn of the rat spinal cord, a marker of the neuroimmune response evoked by morphine repeated treatment. Moreover, PEA restored histamine and methyl histamine plasma levels that were increased by morphine treatment.
CONCLUSIONS: PEA delays the development of tolerance to the analgesic effects of morphine and potentiates opioid efficacy in neuropathic rats. The supplementation of morphine with PEA allows a low dose and long-lasting analgesic effect suggesting the use of PEA for the clinical support of the opioid-based management of persistent pain.

INHIBITION OF MICROGLIA OVER-ACTIVATION IMPROVES BRAIN DEVELOPMENT AND BEHAVIOR IN A MOUSE MODEL OF Cdkl5 DEFICIENCY DISORDER

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OBJECTIVE: CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental disease caused by mutations in the X-linked CDKL5 gene. The consequent misexpression of the CDKL5 protein in the nervous system leads to a severe phenotype characterized by early-onset epilepsy, intellectual disability, and autistic features. To date, no therapies are available for CDD. Evidence in animal models of CDD, that recapitulate various features of the disorder, has shown that absence of CDKL5 negatively affects neuronal survival and dendritic outgrowth; however, knowledge of the substrates underlying these alterations is still limited. Recently, we found increased microglial activation in the brain of a mouse model of CDD, the Cdkl5 KO mouse, suggesting that a neuroinflammatory state, known to cause neuronal dysfunction, may contribute to the pathophysiology of CDD. The present study aimed to evaluate the possible beneficial effect of microglia inhibition on brain development and behavior in a Cdkl5 KO mouse.
METHODS: Cdkl5 KO (+/-) mice were treated for 20 days with luteolin (10 mg/kg), and the effects of this potent microglia inhibitor on neurogenesis, synaptogenesis, and dendritic complexity were evaluated. The effects of this treatment on the motor and memory abilities of Cdkl5 KO mice were also evaluated.
RESULTS: we found that inhibition of neuroinflammation by luteolin increases hippocampal neurogenesis in Cdkl5 KO mice and restores dendritic spine maturation and dendritic arborization of hippocampal and cortical pyramidal neurons, resulting in an amelioration of behavioral performance.
CONCLUSIONS: our findings show that microglia over-activation exerts a harmful action in the Cdkl5-null brain, suggesting that treatments aimed at counteracting the neuroinflammatory process should be considered as a promising adjuvant therapy for CDD.

BET INHIBITOR JQ1 COUNTERACTS HYPER-INFLAMMATORY RESPONSE AND PROTECTS SEPTIC MICE FROM ORGAN DAMAGE

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OBJECTIVE: sepsis is a common and life-threatening condition caused by a dysregulated host response to an infection, responsible for high mortality and morbidity rates, which in turn are driven by organ dysfunction. Among the multitude of triggering mechanisms, acute hyperinflammation has a substantial impact on sepsis-related multiple organ failure (MOF). Recently, an epigenetic regulatory protein known as BRD4, which belongs to the bromodomain and extraterminal domain (BET) family, has been shown to mediate inflammation in several clinical disorders, being poorly investigated in sepsis. Moreover, a powerful BRD4 inhibitor (JQ1) has been shown to reduce macrophages activation as well as decrease inflammation in diseases such as periodontitis. Therefore, we aimed to investigate the potential effect of JQ1 in counteracting the hyper-inflammatory state during experimental sepsis.
METHODS: polymicrobial sepsis was induced by cecal ligation and puncture (CLP) in male, five- month-old C57BL/6 mice (30-35 g). After 1 and 18 hours from the CLP or Sham procedure, mice were randomly assigned to receive either JQ1 (50 mg/kg, s.c) or vehicle. Twenty-four hours after surgery, organs and plasma samples were collected for further analyses.
RESULTS: septic mice treated with vehicle showed systemic inflammation (cytokine storm) by the increased levels of TNF-α, IL-6, IL-1β, IL-17 and IL-10. This hyperinflammation was associated to organ damage, demonstrated by the increased blood levels of ALT, AST, creatinine and lactate, which were paralleled by a high severity score and hypothermia. Those effects were reverted by the treatment with JQ1. Particularly, TNF-α and IL-1β levels were downregulated in the CLP+JQ1 group, while the anti-inflammatory cytokine IL-10 was further upregulated by treatment. JQ1 also prevented septic mice from organ injury by lowering ALT, creatinine and lactate levels, which in turn, attenuated severity score and restored the body temperature. Furthermore, septic mice treated with JQ1 showed reduced activation of the inflammatory pathways p38 MAPK and NLRP3 inflammasome, which were overactivated during sepsis.
CONCLUSIONS: our results suggest a role of JQ1 in counteracting the hyper-inflammatory state induced by sepsis. This study may represent a proof to the use of JQ1 in adjuvant treatments to reduce organ damage during sepsis.

MECHANISMS OF CLEAVAGE AND SECRETION OF PEPITEM, A NOVEL IMMUNO-REGULATORY PEPTIDE

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OBJECTIVE: we have identified an immunoregulatory peptide (PEPITEM) that limits T-cell migration into inflamed tissues. PEPITEM is released by adiponectin-stimulated B-cells and is a cleavage product of 14-3-3ζ protein. We aim to elucidate the mechanism responsible for cleavage and release of PEPITEM from 14-3-3ζ.
METHODS: Peripheral blood lymphocytes (PBL) or B-cells were treated with or without adiponectin (10mg/ml). PBL were also treated with or without matrix metalloproteinase inhibitors. In some cases, B-cells were depleted from PBL and replaced with immortalised Raji B-cells or their conditioned supernatants. *Ex vivo*, lymphocyte migration across cytokine stimulated endothelial cells was assessed using phase-contrast microscopy. Protease expression was measured in B-cells or their supernatants, conditioned with or without adiponectin, using a multiplexed protease array.
RESULTS: matrix metalloproteinases and cathepsins were identified as major proteolytic constituents of B-cells and their supernatants, and addition of adiponectin resulted in a significant

increase in their release. Using a panel of broad spectrum and targeted inhibitors, matrix metalloproteinase 9 (MMP9) was identified as the major enzymatic route of PEPITEM production. Adiponectin-mediated inhibition of lymphocyte migration was lost when B-cells were depleted from the lymphocyte pool and regained by addition of adiponectin-stimulated primary B-cells or adiponectin-stimulated Raji B-cells.
CONCLUSIONS: our results suggest PEPITEM is derived by the activity of MMP9 in the extracellular milieu. Immortalised Raji B-cells are a genetically tractable model for investigating PEPITEM biology. Our results offer a novel understanding of the biochemistry of the PEPITEM pathway, which is required to take full advantage of its translational potential.
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THE LOW-DENSITY LIPOPROTEIN RECEPTOR FUELS MTORC1 ACTIVATION IN CD8 T CELLS

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OBJECTIVE: Activation of T lymphocytes combines functional to metabolic rewiring of cell machinery, including cholesterol homeostasis. Here we evaluated the role of LDLR, as a key regulator of cellular uptake, on T cell biology.
METHODS: Immunophenotypic characterization of T cells from WT and LDLR KO mice was performed in vitro (anti-CD3/CD28) and in vivo (ovalbumin vaccination) coupled to proteomics and WB analysis on isolated T cells. T cells from FH (familial hypercholesterolemia) patients, carrying mutations in the LDLR gene, were tested.
RESULTS: LDLR mRNA expression increased after in vitro activation of CD8, but not CD4 T cells, suggesting a different regulation of cholesterol homeostasis between T cell subsets. Functionally, deficiency of LDLR mainly dampened CD8 *vs*. CD4 activation as demonstrated by in vitro proliferation (- 35%, $p < 0.01$) and INFγ production (-39.6%, $p < 0.01$), and in vivo proliferation and cytokine production (↓IFNγ $p < 0.001$, ↓IL13 $p < 0.01$, ↓perforin $p < 0.05$) after ovalbumin vaccination. Addition of LDL to serum free media increased by roughly 15% ($p < 0.01$) CD8 proliferation in WT but not in KO and in CD4 cells. By proteomic and WB analysis we associated this phenotype to a reduced activation of mTORC1 (pmTOR - 40%, $p < 0.01$) and impaired lysosomal organization (reduced lysotracker and LAMP-1 expression). CD8 T cells from FH patients proliferated less (- 36%, $p > 0.05$) compared to sex- and age-matched controls; in addition, CD8 from FH vaccinated for seasonal influenza were tested in vitro with virus-derived peptides, showing a decreased granzyme production (- 60.3%, $p < 0.01$) compared to CD8 from vaccinated controls.
CONCLUSIONS: LDLR plays a critical role in regulating the immunometabolic responses in CD8 Tcells by fuelling the cholesterol-lysosome-mTORC1 axis.

AN *IN VIVO* STUDY ON THE EFFECTS OF BRD4 PHARMACOLOGICAL TARGETING AGAINST DIET-INDUCED METAFLAMMATION

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OBJECTIVE: metainflammation is a low-grade chronic inflammation associated with obesity, impacting on the onset of type 2 diabetes, dyslipidaemia and related cardiovascular injuries. Recent data suggest epigenetic modifications impact in evoking metabolic derangements by regulating inflammatory genes. However, the effect of pharmacological approaches in interplaying epigenetics and inflammation in diet-induced alterations still need to be clarified. Here we focus on BRD4, member of bromo and extra-terminal domain (BET) proteins which are epigenetic regulators. Recent data point out a role for BRD4 in activating the pro-inflammatory NF-κB and Nrf2 pathways. This study aims to explore the potential effects of a selective BRD4 inhibitor, JQ1, in a murine model of high- fat diet.
METHODS: 22 male 4 weeks old C57BL/6 mice were fed with a control normal diet (ND) or a high-fat diet (HD) for 23 weeks. A subgroup from the HD mice were administered JQ1 (20 mg/kg/day, i.p.) starting from the 20th week of the experiment (HD+ JQ1).
RESULTS: HD fed mice presented a higher body weight compared to ND mice: JQ1 was effective in decreasing body weight. HD mice displayed higher level of fasting blood glucose and insulin and an impaired glucose tolerance; JQ1 reduced insulin resistance, restoring a balanced glucose level. HD mice showed increased serum level of leptin and decreased ghrelin, and higher resistin level, compared to ND mice. JQ1 restored plasma levels of leptin and resistin, while no difference emerged in ghrelin level. Metabolic derangements induced by the HD diet provoked an increase of pro- inflammatory cytokines in HD mice: TNF-α, IL-6 and IL-17 serum levels were higher compared to ND group; JQ1 reduced cytokines concentration, evidencing its emerging role as a modulator in diet- induced inflammation. Moreover, JQ1 decreased white adipose tissue (WAT) increased by HD diet, affecting epididymal and retroperitoneal tissues, hinting JQ1 potential implication on visceral fat deposition.
CONCLUSIONS: JQ1 determined a significant decrease in systemic levels of pro-inflammatory cytokines and a robust reduction in WAT accumulation in HD mice, which were paralleled by beneficial effects on body weight, glucose homeostasis and hormone profile. Overall, data suggest BRD4 as a promising target for contrasting diet-induced metainflammation and adipose tissue metabolic derangements.

ADIPOSE TISSUE DYSREGULATION LEADS TO CHRONIC SYSTEMIC INFLAMMATION

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BACKGROUND: endocrine organs synthesize/secrete hormones that regulate other bodily functions throughout the human body. Recent literature has established that adipose tissue synthesizes and secretes hormones, making it the largest endocrine organ in the body. Hormones released from adipose tissue are referred to as adipokines and include leptin, adiponectin, resistin, and vistatin. This presentation will focus on leptin and adiponectin. These adipokines influence many bodily functions including appetite, insulin sensitivity, inflammation, immunity, and fatty acid oxidation to name a few. In lean individuals, adipose tissue secretes limited amounts of leptin and more generous amounts of adiponectin. In proper proportion, these hormones create a homeostatic environment. Dysregulation of adipose tissue occurs when these hormones are produced disproportionately, and disturbances are seen throughout the body. Nearly all human cells contain receptors for leptin and adiponectin, which have been shown to be important inflammation regulators.
DISCUSSION: leptin tends to have pro-inflammatory actions while adiponectin tends to have anti-inflammatory actions. When adipose tissue is dysregulated leptin and adiponectin production is altered and inflammation is then affected. Adipose tissue continues to produce adipokines but in disproportion. Production of leptin increases and production of adiponectin decreases. This results in an increased inflammatory state despite a lack of precipitous event, which is referred to as chronic systemic inflammation.
CONCLUSIONS: adipose tissue dysregulation can have serious repercussions, such as insulin resistance, increased inflammation, dyslipidemia, increased thrombosis, atherosclerosis, and cellular proliferation. These repercussions in turn lead to increased risk of chronic diseases such as diabetes, cardiovascular disease, hypertension, depression, arthritis, and cancer.

RESOLUTION OF INFLAMMATION VERSUS PROINFLAMMATORY STATUS IN HUMAN ACUTE HEART FAILURE AND CARDIOGENIC SHOCK

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OBJECTIVE: Resolvins D1 (RvD1) and E1 (RvE1) mediate inflammation resolution (Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature.* 2014 Jun 5;510(7503):92-101) (Reina-Couto M, *et al.* Resolving Inflammation in Heart Failure: Novel Protective Lipid Mediators. Curr Drug Targets. 2016;17(10):1206-23), but their role in human acute heart failure (AHF) has not been explored yet. Therefore, we evaluated serum RvD1 and RvE1 profiles in AHF and cardiogenic shock (CS) patients, as well as their correlation with inflammatory status, endothelial dysfunction and prognostic scores.
METHODS: study approved by hospital's Health Ethics Committee. Blood samples collected at days 1-2, 3-4 and 5-8 in AHF (n = 23) or CS (n = 25) patients. Blood donors used as controls (n = 22). RvD1, RvE1, myeloperoxidase (MPO), IL-10, endocan, IL-1β, IL-6 and tumour necrosis factor-α (TNF-α) quantified by ELISA/multiplex immunoassays. C-reactive protein (CRP) and prognostic scores (APACHE II, SAPS II) were also evaluated.
RESULTS: at admission, RvD1 was lower in CS ($p = 0.02$ *vs.* AHF), but RvE1increased with AHF severity ($p = 0.004$ for linear trend), being higher in CS ($p = 0.006$ *vs.* controls). Inflammatory status and endothelial dysfunction increased inpatients' groups, with endocan, IL-10andCRPbeinghigherinCS.During hospitalization, there were no changes in Rvs nor a reduction in the proinflammatory/endothelial biomarkers analysed. Within patients, RvD1 was inversely correlated with endocan and SAPS II while RvE1 was positively correlated with IL-1β, IL-6, TNF-α, MPO and CRP.2
CONCLUSIONS: RvD1 seems exhausted or inactived in CS and related to endothelitis and prognosis, while RvE1 parallels the rise in proinflammatory mediators and clinical/haemodynamical severity.

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ANTI-INFLAMMATORY CYTOKINES EXPRESSION BY γδ+ INTRAEPITHELIAL T LYMPHOCYTES IN UNTREATED CELIAC DISEASE

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OBJECTIVE: γδ+ intraepithelial lymphocytes (IELs) are strongly increased in the epithelium of celiac disease (CD) patients. The expansion of these cells still persist in the intestinal mucosa of CD patients despite gluten exclusion from diet, but their role in the pathogenesis of disease is still unclear. Some evidences in treated CD patients, point towards their potential regulatory role by the secretion of TGF-β.
Different techiniques have been used to analyse γδ+ IELs-specific gene expression profile in CD.
Laser capture microdissection (LCM) has allowed cell-type-specific molecular analysis of tissues, without contamination from surrounding cells.
We aimed to investigate the expression of anti-inflammatory cytokines, such as IL-10 and TGF-β, from γδ+ IELs, isolated by LCM on mirror sections.
METHODS: frozen sections of jejunum were obtained from 10 untreated CD patients. γδ+ IELs and intestinal enterocytes (IEs) were isolated by LCM on mirror sections. RNA from each LCM sample was extracted and, after a retrotranscription step, messenger RNA levels for IL-10 and TGF-β were determined by real-time quantitative reverse transcription PCR (RT-qPCR).
RESULTS: increased gene expression levels of TGF-β were observed in γδ+ IELs compared to IEs ($p < 0.05$). In contrast, a higher expression ($p < 0.0001$) of IL-10 was observed in IEs compared to γδ+ IELs.
CONCLUSIONS: these findings suggest that in untreated CD, γδ+ IELs still retain anti-inflammatory activity, suggesting that such cells are actively trying to downregulate ongoing inflamma-

tion. This work underscores the importance of LCM on mirror sections as a valuable tool to perform cell-type-specific molecular analysis.

COBALT CHLORIDE EXCESSIVE CONSUMPTION SHOWS CARDIOTOXICITY IN MALE WISTAR RATS

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OBJECTIVE: the ionic cobalt is widespread and can be transported by blood in the body, causing adverse effects by the generation of reactive oxygen species. The aim of this research was to describe the changes of myocardial cells during experimental cobalt induced cardiomyopathy in male rats.
METHODS: eighteen 3-6 months old mature male Wistar rats weighing 200-250 g were involved in the experiment. Cobalt chloride (CoCl2) water solution was administered orally for 30 days in low dosage (4 mg/kg) which was considered to be cardiotoxic.
RESULTS: long-term oral administration of cobalt resulted in diminished dietary intake and growth inhibition in the exposed rats. The primary morphological alteration of cardiomyocytes is mitochondrial damage that possibly reflects an enzymatic block of oxidative decarboxylation. Due to that myofibrils of the myocardial cells were affected highlighting that the main cause of myofibril reduction could be a lower oxygen intake in the perinuclear area. Necrosis of cardiomyocytes leads to an inflammatory reaction, proliferation of vascular cells, macrophage infiltration, activation of fibroblasts. Progressive activation of matrix metalloproteinases due to ischemia promotes the development of dilatation and worsening of left ventricular function, which leads to the reduction of the contractile support of myocardial cells and can explain the myocardial dysfunction.
CONCLUSIONS: summarizing the in vivo experiments, it can be stated that severe histotoxic cardiomyopathy occurred in male rats. Knowing structural changes in cardiomyocytes could explain the pathophysiology of the disease and allow a correct therapeutic approach.

MACROPHAGE POPULATION PHENOTYPING SHOWS PREDICTIVE POTENTIAL IN MALIGNIZATION OF *H. PYLORI*-ASSOCIATED CHRONIC GASTRITIS

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OBJECTIVE: currently, gastric cancer is ranked fifth for incidence and third for morbidity with a significant increase in a number of young patients. 80% of patients with gastric cancer have/had a history of *Helicobacter pylori* infection. Tumor-associated macrophages are able to regulate the tumor cell proliferation and can affect the tumor cell dissemination. The study was aimed to assess the predictive potential of the macrophage population immunohistochemical phenotyping in early malignization of *H. pylori*-associated chronic gastritis.
METHODS: gastric biopsy samples of male and female patients aged 48 ± 7.2, infected with *H. pylori* were used as the research material. The patients were divided into three groups: non-atrophic chronic gastritis (NACG, n = 10), atrophic chronic gastritis (ACG, n = 10), G1/G2 gastric adenocarcinoma (GAC, n = 10). The macrophage population was visualized using the CD68 pan-macrophage marker and the type 2 monocyte/macrophage marker CD163. Intensity of neoangiogenesis was defined using the CD31 endothelial marker by assessing the total cross- sectional area of blood vessels.
RESULTS: it was revealed that chronic gastritis was accompanied by the dynamic increase in the size of the general macrophage population with the progression of atrophic and metaplastic processes. According to immunohistochemical study of biopsies obtained from patients with NCG, the CD163:CD68 ratio was 0.67 ± 0.02, and the total cross-sectional area of blood vessels was 3590.92 ± 356.27 μm^2. Atrophic gastritis and adenocarcinoma were characterized by vector redistribution of monocytes/macrophages into the 2nd functional phenotype. The CD163:CD68 expression index in the group with ACG was 0.81 ± 0.04, and in the group with GAC it was 0.88 ± 0.03. Microvascular area was significantly increased in the groups with ACG and GAC, which reflected tumor neoangiogenesis intensification under the influence of M2 monocytes/macrophages.
CONCLUSIONS: the increased expression of CD163 can serve as a predictor of chronic gastritis malignization together with evaluation of the glandular epithelium atrophy and metaplasia degree.

ADENOSINE A3 RECEPTOR (A3AR) AGONIST FOR THE TREATMENT OF BLEOMYCIN- INDUCED LUNG FIBROSIS IN MICE

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OBJECTIVE: the adenosinergic system is involved in disease development, which is characterized by different degrees of inflammation and fibrosis (Jacobson KA, *et al*. Historical and Current Adenosine Receptor Agonists in Preclinical and Clinical Development. Front Cell Neurosci. 2019;13:124). Idiopathic pulmonary fibrosis is a severe disease characterized by inflammation and fibrosis with unknown etiology and aggravated by lack of successful treatments in humans (Sauleda J, *et al*. Idiopathic Pulmonary Fibrosis: Epidemiology, Natural History, Phenotypes. Med Sci. 2018;6(4):110). The purpose of this study was to evaluate the role of A3AR agonist in a murine model of lung fibrosis (Coppi E, *et al*. Uncovering the Mechanisms of Adenosine Receptor-Mediated Pain Control: Focus on the A3 Receptor Subtype. Int J Mol Sci. 2021;22(15):7952).
METHODS: mice were intratracheally injected with bleomycin and for the successively 21 days they were treated with vehicle or different doses of MRS5980, a highly selective A3AR agonist. We investigated the effects of treatments on lung stiffness, studying the airway resistance to inflation; we measured inflam-

matory markers (TNF-α, IL-1β, IL-10, IL-6) and we evaluated TGF-β expression and α-SMA deposition in lungs, indexes of fibrosis establishment.
RESULTS: bleomycin administration increased lung stiffness, TGF-β levels, α-SMA deposition and content of inflammatory markers. On the contrary, MRS5980 attenuated all the analyzed physiological, biochemical, and histopathological markers dose dependently.
CONCLUSIONS: our findings support the proposal that A3AR agonists could have a therapeutic potential in reducing the progression of signs and symptoms of the disease by decreasing inflammation, TGF-β expression and fibrotic remodeling.

EARLY STAGE DIABETIC CRITICAL LIMB ISCHEMIA IS ASSOCIATED WITH ALTERED FREQUENCY AND FUNCTION OF NATURAL KILLER CELLS

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OBJECTIVE: cellular and molecular inflammatory mediators contribute to neuro/vascular pathology in patients with diabetes mellitus, thus they may represent future biomarkers for selecting patients at risk of critical limb ischemia (CLI), a severe peripheral vascular disease leading to amputation, and death. A low frequency and impaired functionality of angiogenic CD34+ hematopoietic stem and progenitor cells (HSPCs) characterize diabetic patients with CLI. In the same setting, we found a high frequency of natural killer (NKs), innate lymphoid effector cells able to acquire angiogenic capabilities, at least in patients with solid tumors. However, no study confirmed changes in the frequency and angiogenic functions of circulating NKs in diabetic CLI.
METHODS: we conducted an observational study on a total of 389 patients divided into 5 groups (nondiabetic control, C = 40; Diabetic, D = 54; Diabetic with Neuropathy, N = 93; Diabetic with Neuro-Ischemia, N1 = 85; Diabetic with Neuro-Vascular Pathology, NV = 117). The frequency of B cells, CD4+ and CD8+ T cells, NK cells, and CD34+HSPCs cells in the peripheral blood was analyzed by multicolor flow cytometry. In a subgroup of C and N patients, NK cells were isolated by FACS-sorting and further characterized for their functional angiogenic activities (matrigel) and lytic abilities (degranulation). Moreover, FACS-sorted NKs conditioned media (CM) were profiled by proteome antibody-membrane arrays.
RESULTS: using logistic multivariate models adjusted for age and sex we confirmed a significant decrease in CD34+HSPCs in all diabetic groups compared to C. Interestingly, we found a significant increase in NK frequency in diabetic patients at an early stage of neuropathy (N) *vs*. C. No change in T and B cells was shown. Next, no change in C and N patients' NK lytic ability was found ($p = 0.08$). Of note, N-NK CM inhibited angiogenesis ($p = 0.0004$). Lastly, secretome analysis of N confirmed significant downregulation of angiogenic factors (*i.e.* CXCL-8, PDGF-BB, FGF-4, *etc.*).
CONCLUSIONS: these results demonstrated an altered frequency and angiogenic function of NKs in early-stage neuropathy that if confirmed in larger cohorts will open to future preventive and treatment care for diabetic CLI.

SNPs OF BITTER TASTE RECEPTORS AS PREDICTIVE MARKER OF ASTHMA

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OBJECTIVE: bitter taste receptors (TAS2Rs) have been demonstrated to be expressed in ectopic tissues such as in the airways. We recently studied TAS2R46 expression, demonstrating its anti-inflammatory role in bronchial epithelial cells (Talmon M, et al. Anti-inflammatory Activity of Absinthin and Derivatives in Human Bronchoepithelial Cells. J Nat Prod. 2020;83(6):1740-50) and its bronchodilator potential in airway smooth muscle cells after a histamine-induced calcium rise (Talmon M, et al. Absinthin, an agonist of the bitter taste receptor hTAS2R46, uncovers an ER-to-mitochondria Ca2+-shuttling event. J Biol Chem. 2019;294(33):12472-82). However, the downstream signalling is still debated. Interestingly, SNPs of TAS2R have been recently linked to altered innate immune responses in some pulmonary diseases (Purnell PR, et al. Single Nucleotide Polymorphisms in Chemosensory Pathway Genes GNB3, TAS2R19, and TAS2R38 Are Associated with Chronic Rhinosinusitis. Int Arch Allergy Immunol. 2019;180(1):72-8). Our interest is therefore to identify in asthmatic patients SNPs in TAS2R46/38 gene sequences looking for a possible correlation with asthma predisposition and drug response.
METHODS: DNA is extracted from patients and controls buccal swabs. SNPs are analysed by TaqMan Assay. SNPs are reproduced *in vitro* by mutagenesis and downstream calcium fluxes evaluated by Fura-2AM and aequorin probes.
RESULTS: absinthin, the specific agonist of hTAS2R46, reduces the cytosolic calcium-rise induced by histamine, increasing the mitochondrial Ca^{2+}-uptake with a TAS2R46-dependent mechanism. SNPs prevalent in the asthmatic population will be correlated to asthma onset, severity of disease and response to therapies.
CONCLUSIONS: all together these data will lead to the target validation and the identification of genetic biomarkers representative of a population more prone to asthma. The presence or absence of SNPs could enable to move toward a personalized therapy, allowing to predict if a patient would respond to a specific bitter agonist or not.

ASSOCIATION BETWEEN C-REACTIVE PROTEIN AND GLYCA INFLAMMATION BIOMARKERS WITH LIPID PROFILE IN ADULTS WITH AND WITHOUT PSORIASIS

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OBJECTIVE: high sensitivity C-reactive protein (hs-CRP) and Glycoprotein Acetylation (GlycA) are biomarkers of systemic in-

flammation associated with lipid profile in general population. However, is unclear whether the association of inflammation biomarkers with lipid profile in adults with Psoriasis (PSO) is similar to those without disease, since PSO is a chronic inflammatory disease with metabolic repercussions. This study aimed to analyze the association of hs-CRP and GlycA with lipid profile in participants with and without PSO from Brazilian Longitudinal Study of Adult Health (ELSA-Brasil).
METHODS: this cross-sectional analysis included participants without coronary disease or use of medicines for dyslipidemia. Hs-CRP, Total Cholesterol (TC), LDL-Cholesterol (LDL-C), HDL-Cholesterol (HDL-C), and Triglycerides (TG) were measured by colorimetric enzymatic method and GlycA was measured by nuclear magnetic resonance. PSO were identified by self-reported medical diagnosis. Covariates were sex, age, ethnicity, educational level, smoking, alcohol consumption, body mass index, diabetes, and hypertension. Median and interquartile range (IQR) values were compared according to PSO by Mann Whitney U test. Inflammation and lipid biomarkers were log10 transformed for data normalization and associations were analyzed by linear regression models.
RESULTS: data from 4,226 participants were analyzed (50.5 ± 8.6 years; 54.6% women) and 107 participants with PSO were identified (3.0%). PSO participants showed higher hs-CRP (1.84 [IQR 4] *vs*. 1.44 [IQR 3], p = .021); GlycA (426.0 [IQR 93] *vs*. 409.0 [IQR: 85], p = .026), and TG (113.3 [IQR: 73] vs. 102.8 [IQR: 73], p = .022) than those without PSO. The association of inflammation biomarkers with lipid profile in participants with PSO was observed only between GlycA and TG (β = .756, p = .015). Among participants without PSO, both inflammation biomarkers were associated with TC (hs-CRP β = .010, p = .002; GlycA β = .197, p < .001); LDL-C (hs-CRP β = .017, p < .001; GlycA β = .230, p < .001); HDL-C (hs-CRP β = -.013, p < .001; GlycA β = -.188, p < .001), and TG (hs-CRP, β = .049, p < .001; GlycA β = .977, p < .001).
CONCLUSIONS: PSO participants had higher concentration of inflammation biomarkers than those without PSO, but the only association of inflammation with lipid profile was observed between GlycA and TG. Further investigation of severity of disease and particle subspecies of lipids are still needed.

LONGITUDINAL ASSOCIATION OF GLYCA INFLAMMATORY BIOMARKER WITH CAROTID AND FEMORAL INTIMA-MEDIA THICKNESS IN BRAZILIAN ADULTS: A 9-YEAR FOLLOW-UP

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OBJECTIVE: Glycoprotein Acetylation (GlycA) is a novel biomarker for systemic inflammation and intima-media thickness (IMT) has been considered as a surrogate marker for subclinical cardiovascular disease. However, longitudinal association of GlycA with IMT measured at different arterial segments in adult population is still not established. The present study aimed to analyze the association of GlycA at baseline with IMT from carotid and femoral arteries after 9-year follow up in adults from Brazilian Longitudinal Study for Adult Health (ELSA-Brasil).
METHODS: data from 3805 participants were analyzed (50.5 ± 8.7 years-old, 55.2% women). GlycA was analyzed by nuclear magnetic resonance at baseline enrollment, while carotid and femoral IMT were measured by ultrasonography in a 9-year follow-up. Covariates were sociodemographic factors (age, sex, ethnicity, educational attainment), adiposity indicators (body mass index, waist circumference), lifestyle (smoking, alcohol consumption), and comorbidities (dyslipidemia, diabetes, hypertension). GlycA and IMT values were log10 transformed for data normalization. Pearson correlation and linear regression were used to analyze association between variables.
RESULTS: the association of carotid with femoral IMT was significant (β = .221, p < .001), but carotid IMT values explained only 9.7% of femoral IMT variance (R^2 = .097), reaching 18.2% in the fully adjusted model for covariates (R^2 = .182). GlycA was associated with carotid (β = .093, p < .001) and femoral (β = .103, p = .002) IMT values, even after mutual adjustment for each other. GlycA remained associated with higher IMT values even after hierarchical adjustment by sociodemographic factors (carotid IMT β = .112 p < .001; femoral IMT β = .139 p < .001), adiposity (carotid IMT β = .069 p = .002; femoral IMT β = .132 p < .001), unhealthy habits (carotid IMT β = .058 p = .009; femoral IMT β = .099 p = .003) and comorbidities (carotid IMT β = .042 p = .056 [marginally]; femoral IMT β = .074 p = .028).
CONCLUSIONS: GlycA was associated with higher IMT values in adults after 9-years of follow-up, especially in femoral artery. The weak association of carotid with femoral IMT suggests that the influence of inflammation biomarkers or other determinants may be heterogeneous at different arterial beds in the adult population.

IMMUNOPEPTIDE AS A NEW THERAPY FOR RHEUMATOID ARTHRITIS

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OBJECTIVES: the inappropriate recruitment and retention of T-cells into the joint is a cardinal feature of RA, yet the molecular mechanisms underpinning this remain unclear. We recently showed that patients with Rheumatoid arthritis (RA) have a defect in a newly identified immuno-protective checkpoint (adiponectin-PEPITEM axis) that normally limits T-cell trafficking during inflammation. Here we examined the therapeutic potential of PEPTIEM in a murine model of arthritis.
METHODS: arthritis was trigger in wildtype mice by immunisation with either bovine type II collagen or methylated BSA. Synthetic PEPITEM was administered by daily injections starting at day 21 (prior to disease onset) or at the first signs of inflammation. Disease onset and severity were evaluated daily. Bone morphology and leukocyte infiltration were assessed by microCT, immunohistochemistry, flow cytometry and qPCR.
RESULTS: administration of synthetic PEPITEM prior to disease onset inhibited the development of arthritis. We observed a significant reduction in disease incidence, clinical score, leukocyte infiltration and bone erosion when compared to control mice. Excitingly, PEPITEM also reduced the clinical score, leukocyte count in the synovium, but not the draining lymph node, and damage to the bone when administered at the first signs of inflammation.
CONCLUSIONS: thus targeting PEPITEM offers an alternative therapeutic approach for treating T-cell mediated diseases, such as RA.

AGE-ASSOCIATED B CELLS FROM EARLY RHEUMATOID ARTHRITIS PATIENTS HAVE A CHEMOKINE RECEPTOR PROFILE ASSOCIATED WITH MIGRATION TO INFLAMED SYNOVIUM

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OBJECTIVE: Rheumatoid Arthritis (RA) is a chronic autoimmune disease characterised by inflammation in the synovium of the joint. A novel subset of B cells, named age-associated B cells (ABC) which are defined as $CD19^{high}CD11c^{+}CD21^{-}$, has recently been discovered and linked with RA. However, a detailed characterisation of these cells in early drug-naïve RA (eRA) is awaited. Our aim is to characterise age-associated B cells from the peripheral blood and synovial fluid of patients who suffer from early and drug naïve rheumatoid arthritis.
METHODS: gene expression of fluorescence-activated cell sorted peripheral blood (PB) B cells from eRA was assessed using a customised Nanostring nCounter Human immunology v2 panel. B cell subsets from PB and synovial fluid (SF) in eRA were characterised by flow cytometry, while cytokine profiles were assessed by an MSD immunoassay following polyclonal stimulation.
RESULTS: transcriptional analysis showed that ABCs have high expression of the cell adhesion marker, CD97, and the inflammatory chemokine receptors, CXCR3 and CX3CR1, while the lymph node homing receptors, CXCR4 and CXCR5, are expressed at low levels. These results were validated at the protein level using flow cytometry. In keeping with this inflammation-homing phenotype, ABC frequency is elevated in the SF compared to that in PB. Phenotypic analysis of ABC demonstrated they have high expression of CD69, Ki67, and MHC class II and co-stimulatory molecules, as well as secretion of T cell skewing cytokines.
CONCLUSIONS: ABCs are an activated and proliferative subset of B cells that display antigen-presenting cell potential. Their high expression of CD97, CXCR3, and CX3CR1 could promote their migration into inflamed synovium. As CD55 and Thy1/CD90 are both ligands for CD97 and are expressed by SFb, ABCs may interact with SFb via this molecule, resulting in functional effects on both cell types that contribute to the pathogenesis of RA. Further work is currently being carried out on the functional role and consequences of ABC interactions in the synovium with a view to identify novel therapeutic targets.

EFFECTS OF BIOMECHANICAL FORCE ON CELLS DERIVED FROM ORAL MUCOSA

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OBJECTIVE: the oral mucosa is exposed to biomechanical force such as occlusal force. In this study, we investigated the effect of human oral mucus-derived cells on biomechanical force.
METHODS: biomechanical force was applied on HO-1-N-1 and HGFs using a hydrostatic pressure apparatus. The expression and production of inflammatory cytokines and growth factors were examined by real-time RT-PCR and ELISA. Biomechanical force -induced intracellular signal transduction via MAP kinase (MAPK) was also examined.
RESULTS: the mRNA expression levels of IL-6, IL-8, EGF and FGF in HO-1-N-1 were significantly higher biomechanical force than those of the control. The mRNA expression level of FGF in HGFs was significantly higher biomechanical force than that of the control. The protein production of IL-6 and IL-8 in HO-1-N-1 was significantly higher biomechanical force than that of the control. The protein production of FGF and IL8 in HO-1-N-1 and HGFs was significantly higher biomechanical force than that of the control. Biomechanical force also increased p-38 phosphorylation and the addition of a p-38 inhibitor significantly suppressed the production of inflammatory cytokines.
CONCLUSIONS: biomechanical force applied to the oral mucosa caused a decrease in the activity of oral mucosal epithelial cells and induced enhancement of inflammatory cytokine production. Also, excessive biomechanical force was suggested to promote the cure of oral mucosa wounds by promoting the production of growth factors in the oral mucosa.

INDOXYL SULFATE AFFECTS INTESTINAL HOMEOSTASIS AND IMMUNE RESPONSE IN MICE REGULATING INFLAMMATORY RESPONSE AND OXIDATIVE STRESS

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OBJECTIVE: Chronic Kidney Disease (CKD) patients suffer from a chronic state of inflammation and of oxidative stress that are proposed to be also linked to many CKD-associated complications. One of the main sources of inflammation in CKD patients is the intestine, where macrophages are also involved both in the modulation of this process and in the immune response.
METHODS: in this study, the effect of Indoxyl sulfate (IS), an uremic toxin poorly eliminated by hemodialysis, on intestinal homeostasis and immune response was evaluated both in mice and in primary murine peritoneal macrophages, but also on intestinal epithelial cells (IEC-6). C57BL/6J mice were treated with IS (800 mg/kg ip), for 3 or 6 hours.
RESULTS: histopathological analysis showed that IS induced intestinal inflammation and increased cyclooxygenase-2 expression (COX-2), nitrotyrosine formation, and the pro-apoptotic protein Bax expression in intestinal tissue. In addition, IS showed an increase in pro-inflammatory and oxidative stress parameters also in mice peritoneal macrophages. The proinflammatory effect of IS also resulted in an increase in mice sera of TNF-α, IL-6, and IL-1β levels. Studies on IEC-6 cells indicated that IS (125-1000 μM) increased the levels of tumor necrosis factor-α and of COX-2 and inducible nitric oxide synthase expression and nitrotyrosine formation.
CONCLUSIONS: taken together, our results indicated that IS significantly contributes to inflammatory and oxidative stress and apoptosis at different levels in CKD by affecting both intestinal homeostasis and inducing a systemic pro-inflammatory state. IS could, therefore, be considered a valid therapeutic target in patients with CKD and its control could be of primary importance in inflammatory and oxidative stress-mediated CKD complications.

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