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PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA

PPGIm
Programa de Pós-Graduação em Imunologia - UFBA
EXPO 2023

XXIII ExpoPPGIm
Reunião Anual do Programa de Pós-graduação em Imunologia UFBA
05 a 07 de dezembro de 2023

Instituto de Ciências da Saúde
Universidade Federal da Bahia – Campus Vale do Canela

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RESUMOS

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SUMÁRIO

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APRESENTAÇÃO

O Programa de Pós-graduação em Imunologia (PPGIm) há mais de 30 anos, vem formando recursos humanos de excelência, capacitados para as atividades de ensino e pesquisa em Imunologia e áreas correlatas, muitos já absorvidos por instituições da Bahia e de outros estados. O PPGIm tem realizado reuniões científicas visando difusão do conhecimento científico e integração acadêmica com a graduação e a pós-graduação da própria UFBA e outras IES. A ExpoPPGIm, Reunião Anual do Programa, já se tornou um evento tradicional, que acontece a cada ano, com a primeira edição no ano 2000, constituindo um fórum de integração de profissionais, pesquisadores e jovens cientistas, alunos de graduação e pós-graduação da UFBA e outras IES do Estado da Bahia, do Brasil e de outros Países com interesse no amplo domínio da Imunologia. O objetivo da ExpoPPGIm é divulgar conhecimento científico em Imunologia e áreas correlatas, gerado localmente, na Bahia, no Brasil, e outros países, tendo como público-alvo estudantes de graduação e pós-graduação, pesquisadores da UFBA e outras IES e profissionais da área. Esta XXIII Edição da ExpoPPGIm que aconteceu entre os dias 05 e 07 de dezembro de 2023 foi realizada nas instalações do Instituto de Ciências da Saúde, local de sede do Programa, e contou com a participação como palestrantes além de pesquisadores da própria UFBA, pesquisadores vinculados a outras instituições de ensino e pesquisa da Bahia e do Brasil, que apresentaram palestras em sessões temáticas, relacionadas às linhas de pesquisa do Programa. Ainda, durante o evento, discentes do Programa apresentaram e discutiram sobre seus projetos de pesquisa em desenvolvimento distribuídos em sessões pôster, assim também contribuindo para a integração acadêmica e a difusão do conhecimento científico em Imunologia e seus correlatos.

ANTI-INFLAMMATORY EFFECT OF *CROTALUS DURISSUS CASCAVELLA* VENON IN EXPERIMENTAL MODELS

Heloyá Vitória de Souza Oliveira, Ellen Caroline Pinheiro da Silva, Rafaela de Oliveira Pinto, Edla Ribeiro Oliveira, Emily Oliveira Cruz, Cailan Carvalho, Rute Soares Nascismento Fraga, Luciana Lyra Casais-e-Silva

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Introduction: The search for new anti-inflammatory therapies is constant, and research with substances of natural origin is of relevant importance. Therefore, natural products are a source of research to be explored. **Objectives:** The present study aims to evaluate the anti-inflammatory potential of *Crotalus durissus cascavella* (Cdc) venom, a subspecies found in the Northeast region of Brazil. **Methodology**: The anti-inflammatory activity of Cdc venom was assessed in different experimental models through pre-treatment at 1h, 2h, or 6h, or immediate administration before the induction of inflammation, subcutaneously, at doses of 50, 75, and 100 µg/kg. Paw edema was induced by administering 15 µg/kg of carrageenan in 50 µL in the right paw of the mouse and an equal volume of sterile saline solution in the contralateral paw, evaluated at various time points (15 min, 30 min, 1h, 3h, 6h, and 24h) using a digital caliper. To assess the antinociceptive ability of Cdc venom, a formalin-induced nociception test was performed using a 2% solution, administered intraplantarly. The assessment was carried out in two phases, 0-10' (neurogenic) and 10'-30' (inflammatory), measuring the time, in seconds, that the animal spent licking, shaking, or lifting its paw. In the following steps, the evaluation of hypernociceptive activity and the action of the venom on leukocyte migration after carrageenan administration will be performed. Cytokine levels in peritoneal lavage will also be quantified using the ELISA method. All results will be compared with a reference anti-inflammatory drug. **Results**: The findings demonstrated that Cdc venom exhibits antiedematogenic activity both in pre-treatments and immediately before carrageenan administration. In the 6-hour pre-treatment, a reduction in edema was observed from 3h, with inhibition up to 24h at doses of 50 and 75 µg/kg. When administered immediately before carrageenan, a noticeable reduction in edema was observed at all administered doses. It was also observed that Cdc venom has antinociceptive activity when administered subcutaneously, albeit only in the inflammatory phase of the test. **Conclusion**: With the results obtained so far, we can conclude that Cdc venom demonstrates anti-inflammatory potential in the experimental models investigated. More studies are needed to understand the mechanisms involved in this activity.

Keywords: Crotalus durissus cascavella; anti-inflammatory; inflammation.

Support: The project does not have specific funding.

ANTITUMOR MECHANISMS OF RUTIN AND IMMUNE MODULATION IN HUMAN GLIOBLASTOMA THROUGH MICROGLIAL INTERACTIONS AND MIRNA-125B REGULATION

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Introduction: Glioblastoma (GBM) is the most aggressive and treatment-resistant brain tumor. Interactions with microglia in the GBM microenvironment lead to dysregulation of cytokines, chemokines, and miRNAs, contributing to angiogenesis, proliferation, anti-apoptosis and chemoresistance. Rutin, a flavonoid, has shown potential in inhibiting rat glioma cell growth by activating microglia and producing pro-inflammatory mediators, but the underlying mechanisms are unclear. Objectives: This study aims to clarify rutin's antitumor and immunomodulatory mechanisms in human GBM cells through interactions with human microglia and their impact on miRNA expression. Methods: We used human GBM GL15 cells and human microglia C20. Cell viability was assessed using the MTT assay in both cell types with and without rutin treatment (1-50 µM) for 24h. miRNA-125b expression in GL15 and their secretome was evaluated 24h post rutin treatment (30 µM) using RT-qPCR. mRNA expression for cytokines IL-1β, IL-6, IL-10, TNF, and the signaling protein STAT3 in C20, treated with conditioned medium from GL15 under control conditions (MCGC) or treated with conditioned medium from GL15 treated with rutin (MCGR), was assessed 24h after treatment via RT-qPCR. STAT3 protein expression in GL15 and C20 under different conditions was examined by Western blot, while cell morphology was analyzed through phase microscopy. Results and conclusion: Rutin treatment (30-50 µM) significantly reduced GL15 viability by approximately 50% after 24h, while C20 viability remained unaffected. Furthermore, rutin treatment of GL15 significantly reduced miR-125b expression and STAT3 protein levels. Conversely, C20 exposed to MCGR exhibited morphological changes suggestive of reactivity and showed reduced mRNA expression for IL-6, TNF, and STAT3, along with decreased STAT3 protein levels. This study reaffirms rutin's potential as an antiglioma agent and highlights its role in modulating miRNA-125b expression. This modulation, through indirect interaction with microglia, likely contributes to altering the inflammatory profile in these cells, promoting a more responsive antitumor phenotype.

Keywords: glioblastoma, microglia, miR125b, rutin, inflammatory cytokines.

Support: CNPq, INCT-Translational Neuroscience, CAPES.

ASSESSING IFN-Γ AND IL-10 RESPONSES TO BCG MOREAU NONPOLAR LIPIDS IN COMPARISON WITH *MYCOBACTERIUM TUBERCULOSIS*

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Introduction: *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB), remains one of the main challenges to public health with were 1.6 million deaths and 10.6 million new cases in 2021. Bacillus Calmette-Guérrin (BCG) is the only licensed vaccine against TB, despite its variable efficacy (0-80%) against the pulmonary from of the disease in adults. Recent clinical trials indicate the importance of exploring diverse classes of antigens to increase the vaccine protection already induced by BCG. Thus, lipid antigens are promising, considering their high concentration in the mycobacterial cell wall and central role in host-pathogen interactions. **Objectives:** This study evaluated and compared the concentrations of IFN-γ and IL-10 levels induced by nonpolar from BCG Moreau and Mtb, at different time intervals, in the supernatants of cultures of PBMC from healthy individuals at different time points. **Material and Methods:** The study was approved and carried out at the Gonçalo Moniz Institute (Fiocruz Bahia, CAAE: 57273322.4.0000.0040). Here, the levels of IFN-γ and IL-10 induced by nonpolar lipids of BCG Moreau and *Mtb* in the supernatant of PBMC cultures from healthy individuals were compared. Nonpolar lipid extracts were obtained from planktonic cultures with the addition of petroleum ether and methanol with 0.3% NaCl (1:10) for cell culture plates sensitization. Culture supernatants were collected to measure IFN-γ and IL-10 levels by ELISA after 24_h, 48_h and 72_h of culture. *Mtb* nonpolar lipid extract induced higher concentrations of IFN-γ and IL-10, unlike BCG Moreau's lipid extract. **Results and Conclusions:** After 24_h and 48_h, *Mtb* lipid extract induced a higher concentration of IFN-γ, when compared to BCG Moreau ($p<0.05$) and NCS ($p<0.001$). Furthermore, nonpolar lipids from *Mtb* induced greater productions of IL-10 in all times-points of cultures evaluated, when compared to NCS ($p<0.05$ and $p<0.0001$). BCG's lipid extract induced only an increase of IL-10 levels after 72_h ($p<0.0001$). The further characterization of the cellular response induced by mycobacterial nonpolar lipids extracts might facilitate future identification of isolated lipids with greater antigenic potential that could be considered as adjuvants in new vaccine schemes against TB.

Keywords: BCG, Tuberculosis, Lipids, IFN-γ, IL-10.

Support: Foundation to Support Research in the State of Bahia (FAPESB).

ASSOCIATION BETWEEN INFLAMMATORY CYTOKINES WITH ANTHROPOMETRIC MEASUREMENTS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS.

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Introduction: COPD is characterized by partially reversible airflow obstruction resulting from chronic inflammation. In stable individuals with COPD, low-grade inflammation is caused by circulating cytokines and influenced by the metabolic activity of adipose tissue. **Objectives:** This study investigates the associations between serum cytokine levels, BMI and triceps skin fold thickness in patients with stable COPD. **Material and Methods:** Patients diagnosed with COPD through spirometry (FEV_1/FVC ratio <0.70), aged between 50 and 80 years. These individuals were recruited from the Respiratory System Service of the Edgard Santos University Hospital (HUPES-UFBA), located in Salvador (Bahia, Brazil). In addition to clinical and nutritional assessment (BMI, arm muscle circumference and tricipital skin fold thickness), serum levels of inflammatory markers (IL-6, IL-8, IL-10, IL-12, and TNF) were measured. This study was approved by the HUPES Research Ethics Committee (Protocol 4.113.435). **Results and Discussion:** Fifty-nine individuals were included, with a mean age of 65.07 (±7.47) years. Of these, 45,7% of the total had a history of exacerbations in the last year, with symptoms of dyspnea averaging 2.12 (±1.23) according to the mMRC scale. The FEV_1/FVC ratio was 53.66(±11.20) % and FEV_1 % predicted was 43.45(±14.16). Furthermore, the patients' anthropometric parameters were evaluated [height= 1.58(±0.11) m; weight= 61.44(±18.06) kg; skin fold= 12.02(±6.87) mm; BMI= 24.97(±7.04) kg/m2]. Regarding cytokines levels, the averages found were IL-6=8.53(±6.48) pg/mL; IL-8=18.64(±12.62) pg/mL; IL-10=6.87(±2.84) pg/mL; IL-12=9.59(±5.13) pg/mL and TNF=10.76(±5.71) pg/mL. Among the nutritional parameters evaluated, tricipital skinfold thickness was positively correlated with IL-6 (p=0.0007; r=0.4963) and IL-8 (p=0.0197; r=0.3466), while no correlation was observed in relation to TNF (p=0.2797; r =-0.1686), IL-10 (p=0.3566; r=-0.1631) and IL-12 (p=0.9417; r=0.01010). **Conclusions:** The fat reserve (tricipital fold) may influence the systemic levels of inflammatory cytokines in stable patients with COPD.

Keywords: COPD; cytokines; adipose

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) 406360/2021-9 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

ATP-BINDING CASSETTE TRANSPORTERS AND DRUG RESISTANCE IN CUTANEOUS LEISHMANIASIS

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Introduction: *Leishmania* spp. are intracellular protozoan parasites causative of cutaneous leishmaniasis (CL). Known as one of the neglected tropical diseases and affecting millions of people around the world, this disease represents a major public health problem. In Brazil, pentavalent antimoniate (SbV) is the main drug used to treat all clinical forms of leishmaniasis, and one of the biggest problems in treating the disease has become treatment failure. ATP-binding cassette transporters (ABC) transporters are exporters of xenobiotics, and its activity has been associated with drug resistance in a variety of diseases. Current evidence shows that some ABC transporters (MRP1 and MDR1) have a role in drug resistance leishmaniasis. The higher activity of these proteins may be associated with therapeutic failure and our hypothesis is that the use of drugs that inhibit ABC transporters in association with SbV may reduce the efflux of the drug in infected macrophages. **Methods:** We used peripheral blood-derived macrophages from healthy individuals infected with *L. braziliensis* strains obtained from lesions of patients with CL that healed and failed treatment with SbV. We verified the activity of ABC transporters MDR1, MRP, and BCRP after infection by L. braziliensis strains and a SbV dose-effect curve directly in promastigotes and amastigotes by flow cytometry. **Results and Conclusion:** The infection by *L. braziliensis* derived from patients with CL was able to increase the efflux of the dye, suggesting a higher activity of ABC transporters after the infection. The administration of MDR1 and MRP inhibitors were able to increase significantly the presence of the dye inside the macrophages, even after infection with *Leishmania* and more studies are being executed by our research group about the association of ABC inhibitors with SbV along the treatment.

Funding: CNPq, CAPES, CAPES/PRINT 41/2017, NIH AI136862

DESCRIPTION OF THE LYMPHOCYTE PROFILE IN POPULATION WITH ACUTE MYELOID LEUKEMIA IN THE STATE OF BAHIA

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Introduction: Acute myeloid leukemia (AML) is a heterogeneous group of hematological neoplasia, caused by the abnormal clonal proliferation of precursor cells of the myeloid lineage. The profile of immune system cells in AML is not completely elucidated in the population of Bahia. Patients with AML can be stratified into favorable, intermediate and adverse prognosis, based on molecular and cytogenetic findings. **Objective**: The aim of the present study was to describe the lymphocyte profile in individuals with Acute Myeloid Leukemia in a population in Bahia. **Methods**: The peripheral lymphocyte subsets were observed in blood of 43 participants, after new diagnosis and before chemotherapy treatment protocol. Different cluster of differenciation markers (CD3, CD4, CD5, CD8, CD19, CD20, CD38, CD45, CD56) were used by FACSCanto II™ flow cytometer and Infinicyt™ program for cell analysis. Prognostic stratification was based on the FLT3 and NPM1 markers using fragment analysis of PCR products. **Results and discussion**: The lymphocyte profiles analyzed are represented by median and percentile (2.5th – 97.5th). Data found profile of lymphocytic cells in the population: B lymphocytes (CD19+): 10.67 (0.19-23.27)%, CD19+: 155.01 (22.37-1089.71) cell/µll; Total T lymphocytes (TL) (CD3+): 79.21 (51.04-95.67) %, 1991.49 (363.84-23807.28) cell/µll; TL CD4+: 42.21 (25.37-64.37) %, 1040.79 (218.93-9684.91) cell/µll; TL CD8+: 28.28 (11.52-53.38) %, 624.17 (82.95-11145.87) cell/µll; NK cells (CD56+): 7.33 (0.55-34.29) %, 154.04 (15.05-1240.83) cell/µll. In comparison with healthy individuals from other studies without AML, no difference was observed between the intervals. Among the study population stratified for prognosis in this work, some differences in T lymphocytes and plasma cells were detected. **Conclusions**: These findings, in association with futher immunological and clinical factors and additional molecular characteristics, may contribute to a better understanding of the role of the immune system in the progression and clinical outcome of patients with this hematologic malignancy.

Keywords: Lymphocyte; Acute Myeloid Leukemia; *FLT3*; *NPM1*

Support: Labimuno; CAPES

DESIGNING A NOVEL MULTI-EPITOPE IMMUNOGENIC PROTEIN

AGAINST *RHODOCOCCUS EQUI* INFECTIONS

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Introduction: *Rhodococcus equi* is an aerobic, opportunistic, and saprophytic bacterium. This microorganism is responsible for causing severe pyogranulomatous bronchopneumonia in equine foals between 1 and 6 months old. This infection has high rates of mortality and morbidity, significantly impacting equine farming both in Brazil and globally. *Several vaccines have been developed in recent decades, but none have shown satisfactory results for large-scale use.* The reverse vaccinology strategy has proven to be an effective and secure method for the design of multi-epitope proteins. **Objectives**: We seek to design an immunogenic multi-epitope protein with potential application in vaccinal formulations against *R. equi* infections. **Material and Methods**: This work was developed using *in silico* approaches based on comparative genomics, subtractive genomics, and immunoinformatics. First, we retrieved total protein sequences from 76 *R. equi* genomes publicly available in the NCBI database. An analysis was conducted to define the core proteome of *R. equi*, and Blastp was used to find the proteins non-homologous to the host *Equus caballus*. The target proteins were selected according to their subcellular localization, antigenicity, essentiality, and virulence properties. Next, an analysis was performed to predict epitope binding affinity to MHC I, MHC II, and B cells. Overlapping epitopes with high binding affinities were combined with small peptide linkers to construct one chimeric protein. Then, we predicted the tertiary structure of this multi-epitope protein and its toxicity, allergenicity, antigenicity, and homology with the equine host. **Results and Discussion**: 2995 proteins were identified in the core proteome and non-host homologs, out of which nine specific proteins were chosen for epitope prediction. These consisted of a multi-epitope protein with 9 MHC I epitopes and 9 MHC II epitopes connected by appropriate linkers. The multi-epitope protein presents a stable structure with 33.44 kDa and 93.4 % residues in the favorable region, according to the Ramachandran plot. It was predicted as immunogenic, without regions toxic and allergenic, and non-homologous to the host. **Conclusion**: Our *in silico* workflow allowed for the design of a potentially immunogenic multi-epitope protein that should be considered in new vaccine formulations against *R. equi* infections.

Keywords: *Rhodococcosis,* recombinant vaccine, reverse vaccinology, bioinformatics

Support: We acknowledge support from CAPES and CNPq.

DEVELOPMENT OF A MULTI-EPITOPE PROTEIN WITH IMMUNOGENIC POTENTIAL AND PROSPECTION OF NEW DRUGS AGAINST INFECTIONS CAUSED BY *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

Cintia Sena Carvalho, Vitória Monise Santos de Aquino, Sandeep Tiwari, Roberto Meyer, Núbia Seyffert, Thiago Luiz de Paula Castro.

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Introduction: *Corynebacterium pseudotuberculosis* is a Gram-positive and intracellular facultative pathogen known to cause diseases of veterinary importance. Among these, Caseous Lymphadenitis (CLA) is a disease that affects goats and sheep, generating abscesses in the lymph nodes and internal organs, causing economic losses for the breeders of these animals. Prophylaxis is the most cost-effective alternative for controlling infectious diseases, however, there is still no effective vaccine against CLA. Several in silico methodologies have contributed to the selection of molecular targets that can be explored in the development of therapeutic strategies, diagnostic methods, and recombinant immunogens. **Objectives:** Therefore, the present work aimed to select vaccine and therapeutic targets, as well as to construct a potentially immunogenic chimeric protein and to screen out compounds that could be used in the treatment of CLA. **Methods:** Based on data from the literature, the reactive proteins RpfB, SlpA, Nlpc/P60 and the virulence factors CP40 and PLD were selected and evaluated for their intraspecific conservation and homology with proteins from goat and sheep hosts. Subsequently, epitopes of MHC-I, MHC-II and B cells present in these proteins were predicted and evaluated. In addition, proteins shared by all *C. pseudotuberculosis* strains, both cytoplasmic and essential, were selected for virtual screening and molecular docking analysis with natural compounds. **Results and Conclusions:** A chimeric protein was constructed from the epitopes of RpfB, SlpA, Nlpc/P60, CP40, and PLD proteins. This immunogenic potential proved to be stable, soluble, antigenic, and non-allergenic in silico analyses. In the therapeutic approach, the proteins WP_013241317.1, WP_013241598.1, WP_014522829.1, WP_013242887.1, WP_013241937.1 and WP_013241997.1 were selected and natural compounds ZINC04258889, ZINC04235924, ZINC04236001, ZINC04235972, ZINC08300419 and ZINC67902338 were the best binders for them, respectively. These results will contribute to the prophylaxis and treatment of CLA as well as other diseases caused by *C. pseudotuberculosis*.

Keywords: corynebacteria; immunoinformatics; virtual screening; vaccination; treatment.

Support: CAPES

DISTINCT CD105 (ENDOGLIN) EXPRESSION ON NORMAL BONE MARROW B CELLS AND LEUKEMIA BLASTS IN CHILDHOOD B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction: B-cell precursor Acute Lymphoblastic Leukemia (BCP-ALL) corresponds to about 80% of ALL, mainly affecting children and adolescents. Relapses affect about 20% of patients, with 40-70% overall survival after recurrence, highlighting the need for new therapeutic approaches. The CD105 molecule (endoglin) is a TGF-B co-receptor, its expression in BCP-ALL blast cells is related to a poor prognosis. In this context, the analysis of CD105 expression in normal and neoplastic lymphoid precursors can be an important tool to understand the role of this molecule. In addition, it can contribute to monitoring Measurable Residual Disease (MRD) analysis. **Objectives:** To evaluate CD105 expression on normal B cell precursors, mature B cells, and leukemic blasts from pediatric patients with BCP-ALL. **Material and Methods:** Bone marrow (BM) samples from patients diagnosed with BCP-ALL, aged between 0 and 18 years, treated at Hospital Aristides Maltez (HAM) were analyzed. Samples were previously evaluated by flow cytometry for diagnostic purposes (DO) and MRD follow-up (D33). Panels of antibodies that meet the classification of the World Health Organization (WHO) were used, with the inclusion of the anti-CD105 antibody. **Results and Conclusions:** We evaluated CD105 expression in normal B cell precursors, mature B cells, and leukemic blasts from pediatric patients with BCP-ALL. CD105, at diagnosis, was expressed in 73.33% of patients with BCP-ALL, with the highest expression in leukemic blasts. When compared with DRM, the highest expression was in the hematogonia in negative DRM (67.28%), median fluorescence intensity (MFI) of 87.26. When analyzed individually (monitoring from DO to relapse of one patient), the blasts had a higher expression and MFI, 21.56% and 26.14, respectively. We observed positive CD105 expression in 33% of the included patients, with higher population expression and high MFI. Furthermore, we observed a higher expression of CD105 in more immature B cells, suggesting a correlation with the maturation process of the B lineage.

Keywords: BCP-ALL; Maturative curves; Endoglin.

Support: Own financing. Immunophenotypic diagnostic and MRD laboratory data were used in the present work.

ELUCIDATING MOLECULAR MECHANISMS: EXPLORING THE ANTI-GLIOMA AND IMMUNOMODULATORY EFFECTS OF FLAVONOIDS THROUGH AHR INTERACTION

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Introduction: Conventional therapy for glioblastoma (GBM) typically involves surgical resection, radiotherapy, and chemotherapy. However, the tumor's aggressive characteristics, marked by rapid proliferation and the induction of immunosuppression, significantly contribute to its unfavorable prognosis and high recurrence rates. Additionally, the aryl hydrocarbon receptor (AhR), a transcription factor constitutively activated in tumor cells, has been implicated in chemo – and immunoresistance. AhR activation not only promotes cell differentiation but also upregulates the expression of resistance genes, making AhR antagonism an attractive target for cancer chemotherapy. The proven antitumor and immunomodulatory properties of flavonoids highlight their pharmacological potential in GBM treatment. Flavonoids, acting as AhR antagonists, have the potential to decrease the viability of tumor cells. **Objectives:** This study aims to elucidate the antitumor mechanisms of the flavonoid naringenin and explore its potential association with AhR antagonism in anti-glioma activity**. Methods:** Naringenin was assessed as an AhR antagonist at escalating non-cytotoxic concentrations (5-30µM), utilizing the induction of CYP1A1-mediated EROD activity assay in MCF7 cells as an indicator of AhR responsiveness. Human U87 GBM cells were exposed to naringenin (30µM) in the presence or absence of the AhR agonist Indole-3-carbinol. After 24 hours of treatment, cell viability was evaluated using MTT and SRB assays, while cell migration was assessed up to 48 hours post-treatment. **Results and conclusions:** The results demonstrate the potent inhibitory effects of naringenin on AhR activity. Furthermore, the data revealed that naringenin reduced cell viability and migration in a dose – and time-dependent manner. Importantly, the combination of naringenin and the AhR agonist increased cytotoxicity to GBM cells. Characterizing the molecular mechanisms of naringenin, particularly its antagonistic effect on AhR, and understanding its role in chemosensitivity will contribute to supporting its application as an adjuvant in GBM treatments.

Keywords: glioma; glial cells; flavonoids; aryl hydrocarbon receptor; immunomodulation

Support: CAPES, CNPq and FAPESB.

ENVIRONMENTAL FACTORS AND CYTOKINES ASSOCIATED WITH MOOD DISORDERS IN CHILDREN AND ADOLESCENCE

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Introduction: Behavior disorders are characterized by changes in cognition and emotional or behavioral regulation. childhood and adolescence represent a key period for the establishment of brain regulatory patterns and it is possible to observe the development of internalizing and externalizing behaviors. At this stage, socio-environmental and immunological factors were shown to be related to behavioral changes. **Objectives:** The present study pretends to identify children and teenagers with signs of internalizing and externalizing behavior disorders in a population sample and to test their associations with sociodemographic, environmental factors, and cytokine levels in the peripheral blood of individuals. **Methods:** A total of 1,364 children in this case control, recruited by the SCAALA project (Social Changes, Asthma and Allergy in Latin America) were included in the present study. For evaluation of behavior, used CBCL-Child Behavior Checklist" (2005), and the "YSR-Youth Self-Report" (individuals over the age of 11, in 2006). For evaluation of discipline applied and the caregiver's mental distress, the "CTSPC-Conflict Tactics Scales: Parent-child Version" (2006), and the "SRQ-20-Self-Report Questionnaire" (2013). Cytokines (IL-10, IL-5, IL-13, and IFN-γ) from peripheral blood were measured by ELISA and categorized into immunophenotypes according to Figueiredo *et al.,* 2013. **Results:** Normality was tested on children internalization (N=487/35.7%), children externalization score data (N=473/ 29.4%), adolescent internalization (N=85/8.3%), and adolescent externalization (N=404, 39.6%) score data (normal distribution, cut off=64) and cytokine data (non-normal distribution). The $\chi 2$ test revealed an association between internalization and externalization and socio-environmental factors, such as: monthly family income ($P_{children\_externalization} \leq 0.5$) ; Sex ($P_{children\_internalization} \leq 0.5$; $P_{adolescent\_internalization} \leq 0.5$; $P_{adolescent\_externalization} \leq 0.5$) ; maternal schooling ($P_{children\_externalization} \leq 0.5$), violent abuse ($P_{children\_internalization} \leq 0.5$; $P_{children\_externalization} \leq 0.5$), non-violent abuse ($P_{children\_internalization} \leq 0.5$; $P_{children\_externalization} \leq 0.5$), SRQ-20 ($P_{children\_internalization} \leq 0.5$; $P_{children\_externalization} \leq 0.5$) and nonviolent discipline ($P_{children\_internalization} \leq 0.5$;

Pchildren_externalization ≤ 0.5. These results suggest that social factors are important for characterizing behavioral outcomes. Cytokine data was categorized into immunophenotypes, and demonstrated association with children internalizing symptoms (Pchildren_internalization = 0.5). **Conclusions:** Socioenvironmental and immunological factors are associated with childhood and teenagers behavior outcomes in our population, which corroborates other findings in the literature. Less subjective markers such as these may, in the future, be considered auxiliary for the diagnosis of behavioral disorders.

Keywords: Behavior disorders, immunophenotypes, cytokines, socio-environmental components, intra-family violence.

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EVALUATION OF RUTIN'S EFFECT ON GLIAL MARKERS IN THE HIPPOCAMPUS IN AN IN VIVO MODEL OF AMINOCHROME-INDUCED PARKINSON'S DISEASE.

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Introduction: Recent studies on Parkinson's Disease indicate a significant increase in global diagnosis. Mediated by astrocytes and microglia, neuroinflammation plays a crucial role in the progression of the disease. There are indications that the microglia in the M1 profile and the astrocytes in the A1 profile, may contribute negatively to the induction of neurogenesis, while the M2 and A2 cells may develop a pro-neurogenic role. Research suggests that the flavonoid rutin has the potential to reduce cell reactivity and provide neuroprotective properties in aminochrome-induced Parkinson's models. However, further investigations are needed to understand its potential in altering cellular profiles associated with neurogenesis. **Objectives**: To evaluate the effect of rutin on the glial activation status and its relation to neurogenic potential. **Methods:** A study was conducted with 24 male Wistar rats, divided into four groups (CT, RUT, AMI, AMI+RUT), and subjected to stereotaxic surgery in the striatum region with the application of saline in the CT and RUT groups and aminochrome in the AMI and AMI+RUT groups. After 22 days, the brains were prepared for immunofluorescence using antibodies targeting GFAP, S100b, DXC, IBA1, CD68, CD206, and NeuN. **Results and Conclusions:** The immunophenotypic profile analysis of the of astrocytes and microglia in the rutin-treated groups showed that the flavonoid is capable of increasing the density of GFAP+ and S100b+ cells, along with a numerical rise in CD68+ microglia. This result was followed by a numerical decrease in DXC+ and CD206+ cells in the same group. The AMI group did not show alterations in the tested markers. The AMI+RUT group assessment reveals a numerical decrease in DXC+ and CD206+ cells, with an elevation of CD68+ cells. The NeuN marker showed no changes in the tested groups. When administered at the striatal level, aminochrome did not cause alterations in the hippocampus; however, orally administered rutin led to changes in this anatomical region.

Keywords: Neurogenesis; Parkinson's; Flavonoid.

Support: CNPQ, FAPESB.

EVALUATION OF THE ALLERGENIC POTENTIAL OF THE HOUSE DUST MITE GLYCYCOMETUS MALAYSIENSIS

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Introduction: Dust mites are considered one of the main sources of aeroallergens and are associated with the development of allergic diseases. Recurring contact with the allergen can lead to chronic inflammation, which may result in the onset of allergic diseases such as asthma, rhinitis and atopic dermatites. In Brazil, the most prevalent mites belong to the genera *Dermatophagoides* and *Blomia*. However, in a previous study conducted by our group we identified for the first time the presence of the mite *Glycycometus malaysiensis*, which had already been reported as a sensitizing agent in Asian countries. Both *G. malaysiensis* and *B. tropicalis* mites exhibit morphological similarities, raising the possibility of cross-reactivity between their allergens. Nevertheless, the specific allergens of this mite species have not been fully characterized. Objectives: Identification and isolation of the mite *G.malaysiensis* from house dust and evaluation of antigen reactivity in serum from atopic patients. **Material and Methods:** Collection of house dust mites and mite cultures; molecular characterization of mite cultures using PCR; production of mite protein extracts; protein evaluation of extracts using SDS PAGE; and evaluation of extracts using serum from allergic patients (ELISA and Western Blot) were performed. **Results and Discussion:** Using molecular identification we observe: 1 – two masses containing *B. tropicalis* and *D. pteronyssinus* isolates; 2 – two masses containing *D. pteronyssinus* and *G. malaysiensis* isolates; and 3 – one mass containing *B. tropicalis* and *G. malaysiensis* isolates. Then, we evaluated the band patterns of each extract by SDS-PAGE and was observed in both Western-blot with and without inhibitors that patients allergic to *B. tropicalis* and *G. malaysiensis or* allergic to *B. tropicalis* and *D. pteronyssinus* have both positive reactivity to the bands referring to the *G. malaysiensis* mite. In addition, using ELISA technique, we observe that the patients had a higher IgE reactivity to the extract containing *B. tropicalis* compared to the extract containing *B. tropicalis* and *G. malaysiensis*. On the other hand, the extract containing *D. pteronyssinus* had less IgE reactivity in relation to extract containing *D. pteronyssinus* and *G. malaysiensis.* **Conclusions:** In this study, we could observe the immunogenic potential of *G. malaysiensis* and its possible significance in the population tested.

Keywords: *G. malaysiensis;* house dust mites; allergies

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EVALUATION OF TYPE 1 INTERFERON, RECEPTORS IFNAR1/2, INTERLEUKIN 17 A AND ENDOGENOUS RETROVIRUS (HERV-K AND HERV-W) EXPRESSION IN PATIENTS WITH COVID-19

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Introduction: The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in high morbidity and mortality worldwide. The severity of COVID-19 was associated with impaired production of type I IFNs, as well as dysregulation of the expression of the cytokine IL17A, important for the development of COVID-19. Moreover, the activation of human endogenous retroviruses (HERVs) may be related to severity of COVID – 19, but their contribution to COVID-19 severity is still under investigation. **Objective:** To analyze the expression of IFN-α genes and their receptors (INFAR1/INFAR2), IL-17A and HERVs (HERV-K /HERV-W) in the peripheral blood of patients with mild and severe forms of COVID-19. **Methods**: A cross-sectional case study, with 117 patients with a diagnosis confirmed by qRT-PCR, of which 59 participants in the case group and 58 in the control group. We collected whole blood and performed the isolation of mRNA from total leukocytes. The RT-qPCR assay was performed to analyze the relative expression of genes ($2^{-\Delta T}$). **Results:** Among the sample studied, 65.5% of the patients were male with a median of 62 years in patients in the severe group (p<0.0001). Considering comorbidities, the patients showed the presence of at least one pre-existing chronic disease, with diabetes being the most common comorbidity present in 27 patients (46,6%) (p<0.0001). Patients with severe COVID-19 had significantly decreased levels of red blood cells, hemoglobin and percentage of hematocrit, as well as lymphopenia and neutrophilia (p< 0,001). Inflammatory markers such as NLR and PLR were significantly higher in severe patients when compared to mild patients (p< 0.001). It was observed that markers of systemic immuno-inflammation such as, SII, AISI and SII/Hb were significantly increased in patients with the severe form of COVID-19 (p< 0,001). Severe patients showed a significant increase IFNA1, IL17A and HERVW-1 (p<0,05). ROC analysis for genes demonstrated area under curve (AUC), sensitivity and p-value (0.7 ; 100% ; p<0.05, respectively) for IL-17A resulting in positive diagnostic value. The NLR demonstrated better diagnostic value when compared to the PLR (AUC: 0.978;0.896; p<0.0001, respectively). ROC analysis for indices SII,AISI and SII/Hb demonstrated area under curve (AUC) (0.9 ;0.6 ;0.9;p<0.05, respectively) suggesting potential biomarkers for COVID-19. **Final Considerations:** Overall, our study demonstrated that the segment most affected by COVID-19 comprises males, the elderly, black people with diabetes and the high expression of IFNA1, IL17A and HERVW-1 in patients admitted to the intensive care unit, may be a reflection of the exacerbated immune response. Together the findings suggest that NLR, PLR and markers systemic immuno-inflammation such as, SII, AISI and SII/Hb have a potential relationship with predicting the outcome of COVID-19.

Keywords: COVID-19. INFAR1. IL-17A.

Support: FAPESB, CNPq and PIBIC-UFBA.

EXPRESSION OF RECOMBINANT PROTEIN FROM *TOXOCARA CANIS*

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Introduction: T. canis and T. cati are gastrointestinal nematodes of the genus Toxocara spp. which complete their life cycle in the digestive tract of dogs and cats, and in paratenic hosts, including man, this cycle cannot be completed and the larvae remain in the host, passing between organs and systems. Toxocara spp., infection triggers immune responses from the innate immune system, activating the adaptive system and consequently producing Th2 cytokines that cause eosinophilia and an increase in IgE. Currently, toxocariasis is diagnosed using the larval excretory secretory antigens of this nematode (TES), but the cross-reactivity of antibodies (IgG or IgG4) with antigens from other helminths can lead to a false positive diagnosis. With advances in technology and molecular biology, it is possible to produce recombinant molecules to improve immunodiagnostic techniques, making tests more sensitive and specific for antibodies against specific antigens. **Objectives:** To evaluate the possible cross-reactivity of the FULL protein, a T. canis specific fusion protein (TES-26 and CTL-4), in the different immunodiagnostic techniques using sera from patients with different parasitic infections. **Methods:** A synthetic plasmid containing the FULL sequence was transformed into different strains of E. coli, and expression of the heterologous protein was induced with IPTG for 4 hours. The bacterial extracts were solubilized in a pH 9 buffer solution and purified using affinity chromatography. The expression and purification of the protein were confirmed by Western Blot. Finally, the reactivity of the protein was evaluated with serum from asthmatic patients using Dot Blot and indirect ELISA. **Results and Conclusions :** The FULL chimeric protein was constructed with all the probable B-cell epitopes found through the genetic sequences of two molecules (rTES-26 and rCTL4), resulting in a 38 kDa protein. The expression of the protein was observed and confirmed by western blotting after 4 hours of expression in the bacterial culture, and affinity purification experiments were also carried out using the histag inserted at the C-terminal end of the protein. Through Dot Blot and indirect ELISA, it was possible to confirm the slight immunogenic potential of the FULL protein, which has considerable sensitivity and specificity when compared to TES for future use in immunodiagnosis.

Keywords: Toxocariasis, Immunodiagnosis, recombinant proteins, Toxocara spp. **Support:** PIBIC-UFBA.

GENETIC SCREENING OF AUTOINFLAMMATORY GENES IN LATIN AMERICAN CHILDREN WITH MULTISYSTEM INFLAMMATORY SYNDROME (MIS-C) ASSOCIATED WITH SARS-COV-2 INFECTION

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Introduction: Multisystem Inflammatory Syndrome in Children (MIS-C) is an inflammatory condition associated with SARS-CoV-2 infection. It is characterized by fever, prominent gastrointestinal symptoms, mucocutaneous manifestations, respiratory symptoms, and patients often present with shock. The occurrence of MIS-C may be associated with congenital defects of immunity. **Objectives:** identify variants in genes involved in primary autoinflammatory conditions that may be implicated in MIS-C; **Methods:** Cells and clinical and laboratory information were collected from 21 pediatric patients with MIS-C, recruited from three public hospitals in the Northeast region of Brazil. The cases of MIS-C were classified as severe or moderate, considering, respectively, the need or not for Positive Pressure Ventilation (PPV) and/or vasopressor medication. Then, total exome sequencing (WES) of the individuals was performed and the identified Individual Nucleotide Variants (SNVs) were subjected to an Inborn Errors of Immunity (IEI) prioritization strategy, focusing on 56 genes previously implicated in auto-inflammatory diseases (made available by the Immunity Committee of the International Union of Immunological Societies, 2022). **Results and Conclusions**: Six SNVs were identified in 5 different genes (*ADAM17, CARD14, IKBKG, PSTPIP1* and *SH3BP12*). All these variants have been found in children/adolescents with the severe form of MIS-C. Notably, two variants (rs1200631089 and rs144458353) were selected in the *ADAM17* gene. This gene encodes a protease implicated in the processing of tumor necrosis factor alpha (TNF-α) and plays a key role in SARS-CoV-2 infection by cleaving the Angiotensin-Converting Enzyme 2 (*ACE*-2), the main human receptor for SARS-CoV-2. Our data suggest that rare deleterious variants in genes previously implicated in autoinflammatory conditions, including *ADAM17, IKBKG, PSTPIP1, SH3BP2* and *CARD14*, may explain the occurrence of SIM-P in previously healthy Latin American children and adolescents.

Keywords: MIS-C; SARS-CoV-2; Autoinflammatory; Genetics.

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) and Fundação Oswaldo Cruz (Fiocruz)

GENETIC SIGNATURES OF AKT1 VARIANTS PREDICT WORSE COVID-19 OUTCOMES – A MULTICENTRIC OBSERVATIONAL STUDY

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Introduction: The COVID-19 pandemic, resulting from the global spread of the SARS-CoV-2 infection, was a public health crisis. Approximately 15% of those infected are estimated to progress to severe pneumonia, and about 5% eventually develop acute respiratory distress syndrome due to cytokine storm. PI3K/Akt/mTOR is an intracellular signaling pathway that could play a major role in the inflammatory response and disease severity. Inhibition of *AKT* can potentially suppress pathological inflammation, cytokine storm, fibroproliferation, and platelet activation associated with COVID-19. Objectives: To investigate the rs2494746 and rs1130214 variants in the *AKT1* gene associated with adverse COVID-19 outcomes. **Methods:** Peripheral blood samples and sociodemographic data were collected from 508 individuals with COVID-19, 216 mild cases and 292 severe cases from April 2020 to April 2021. Plasma cytokine concentrations were measured using ELISA. Genotyping of SNPs rs1130214 and rs2494746 were performed using kits from Thermo Fisher and analyzed by qRT-PCR. **Results:** The rs2494746-C allele was associated with severity, ICU admission, and death from COVID-19. The C allele at rs1130214 was linked to increased TNF and D-dimer levels. Moreover, both variants exhibited an increased cumulative risk of disease severity, ICU admission, and mortality caused by COVID-19. In the predictive analysis, the rs2494746 obtained an accuracy of 71%, suggesting a high probability of the test determining the severity of the disease. **Conclusions:** The present study contributes to understanding the influence of the *AKT1* gene variants on the immunological damage in individuals infected with SARS-CoV-2. These findings could be useful in the future to assist in predicting a worse outcome of COVID-19.

Keywords: AKT1, severity of COVID-19, polymorphism.

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GENETIC VARIANTS IN THE TNF PATHWAY IMPACT TNFI RESPONSE IN A MIXED POPULATION WITH RHEUMATOID ARTHRITIS.

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Introduction: Rheumatoid arthritis (RA) is a multifactorial autoimmune inflammatory disease that mainly affects the joints, leading to reduced functional capacity and impact on quality of life. Cytokines such as tumor necrosis factor (TNF) and interleukin 6 (IL-6) are crucial in the pathogenesis of this disease, which has made TNF inhibitors (TNFi) therapy a great advance in its treatment. However, a significant portion of patients do not respond or lose their response to these medications. In Brazil, there is still a lack of studies on the impact of genetic factors on RA. **Objectives:** To evaluate the association of genetic variants in the TNF pathway with the diagnosis of RA and the response profile to TNFi immunobiologicals in patients followed up at public infusion centers in the state of Bahia, Brazil. **Methods:** This is an ambispective cohort study. Subjects diagnosed with RA and healthy controls were included in the study. Clinical, sociodemographic, and genetic data were used to evaluate the associations of variants in *TNF*, *TNFRSF1A*, and *TNFRSF1B* genes with the diagnosis of RA, standardized score results, laboratory tests, and response to TNFi. In one subsample, serum levels of TNF and IL-6 cytokines were performed. **Results and Conclusions:** A total of 360 healthy controls subjects and 294 diagnosed with RA were included in the analysis. Higher levels of TNF have been found in diagnosed with RA. IL-6 levels were higher in individuals who did not respond to TNFi treatment, while responders had levels comparable to the group without the disease. No associations were found between the SNPs studied and the diagnosis of RA, however, rs767455-C seems to play a role in the response to golimumab treatment, being related to better therapeutic response and lower mean serum leukocyte levels. In addition, rs1061622-G was associated with poorer functional capacity and rs1800629-A was associated with both higher leukocyte values and higher serum transaminase levels. Our results highlight the importance of the analysis of these variants in a larger population, as well as other polymorphisms, regarding the diagnosis of RA and response to immunobiological treatment.

Keywords: Immunobiological treatment, TNF, rheumatoid arthritis, polymorphisms, TNFi.

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES

GENOMIC ANALYSIS OF CAMPYLOBACTER SPP. APPLIED TO THE DEVELOPMENT OF A POTENTIALLY IMMUNOGENIC CHIMERIC PROTEIN

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Introduction: Campylobacter is a Gram-negative bacterium of a zoonotic nature and is mainly responsible for causing gastroenteritis in humans through the consumption of contaminated food and water, as well as causing abortions in the fetuses of goats, sheep, and cattle. The World Health Organization has categorized Campylobacter as a high-priority pathogen due to the development of antibiotic resistance and the lack of vaccination. **Objectives:** To explore the genomic information available for different Campylobacter species to propose a potentially immunogenic chimeric protein that could be tested in the future in a new vaccine formulation. Materials and Methods: Bioinformatics tools were used to carry out this work. Data mining was conducted to identify what had already been produced related to subtractive genomics for Campylobacter. The genomes of the chosen species were analyzed to depict phylogenetic features regarding the selected species. **Results and Discussion:** Data mining identified three Campylobacter species most frequently isolated and reported in humans and animals: Campylobacter jejuni, Campylobacter fetus, and Campylobacter coli. Campylobacter fetus presents three distinct subspecies: fetus, verenealis, and testudinum. We noted the genomic features of Campylobacter fetus have yet to be explored as the other species. Thus, 30 complete genomes of this species were obtained from the NCBI database and analyzed, of which 15 belonged to Campylobacter fetus subsp. fetus (7 identified using Genome Similarity Index Analysis – GSA), 5 were Campylobacter fetus subsp. testudinum, and 10 were Campylobacter fetus subsp. verenealis. **Conclusion:** The genomes of Campylobacter fetus from the three subspecies will be used in the following steps to retrieve target proteins that might be considered for epitope prediction and the development of a novel immunogenic chimeric protein against Campylobacter infections.

Keywords: Reverse vaccinology. Immunogen. Genomic analysis.

Support: We acknowledge support from CAPES and CNPq.

IMMUNE RESPONSE OF CORONAVAC SARS-COV2 VACCINATION FROM AN INDIGENOUS BRAZILIAN PEOPLE

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a significant increase in infections and deaths worldwide, making an effective immunization program crucial to protect, especially the most vulnerable people. The dynamic of adaptive immune responses elicited by the inactivated virus CoronaVac™ vaccine was elucidated. However, in indigenous populations its remain elusive. **Objectives:** This study aims to evaluate the humoral and cellular immune responses to the CoronaVac™ vaccine in Brazilian indigenous immunized. **Material and Methods:** In eligible patients who received complete vaccination schedule of CoronaVac™, we analyzed SARS-CoV-2-specific humoral and cellular immune responses at two different timepoints, before vaccination (T1) and 45 days after complete vaccination schedule (T2). SARS-CoV-2-specific antibodies, peripheral B cells, $CD4^+$ and $CD8^+$ T cells and their memory subsets were simultaneously measured in this cohort. A self-report questionnaire interview was conducted to investigate demographic characteristics and factors related to vaccine immunogenicity. Data were analyzed using descriptive statistics, Fisher's exact test and Mann-Whitney test. **Results and Discussion:** We enrolled 328 patients, including 120 (36·6%) patients without SARS-CoV-2 antibodies. Peripheral blood mononuclear cells (PBMCs) were collected from 106 patients during the follow-up visits, of which 91 were analyzed by immunophenotyping assay to detect the SARS-CoV-2-specific memory T-cell responses. A significant increase in IgG antibodies against the Spike protein as well as memory B cells and Natural Killer T lymphocytes were observed 45 days after vaccination. However, no significant differences were observed in CD4+ T lymphocytes. According with results, our sample was presented a mean age of 36 years, 78% female, 70% relied on government benefits, 32% tobagists and 20% alchoolists. Influenza vaccination was reported in 93% of the cases. Residents of Bororó village, female, and those who identified as Terena ethnicity had higher concentrations of antibodies against SARS-CoV-2 after vaccination, while alcohol and tobacco users had lower concentrations. To the best of our knowledge, this is the first comprehensive evaluation on the antibody and T-cell responses against the CoronaVac™ vaccination in indigenous patients. Our findings show that robust antibody and T-cell immunity against SARS-CoV-2 is present in the majority of patients 45 days after vaccination.

Key words: SARS-CoV-2; vaccine; immune responses; CoronaVac™.

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

IMMUNOHAEMATOLOGICAL PROFILE AND ANCESTRY INFORMATIVE MARKERS IN INDIVIDUALS WITH COVID-19

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Introdution: COVID-19 is characterized by a wide spectrum of clinical manifestations and immune responses can favor unfavorable outcomes. Some ethnic minority groups appear to be more vulnerable to infection or present serious clinical conditions, whether due to biological or sociodemographic factors. There are few data related to genomic ancestry and clinical outcomes of the disease, for this reason, **Objective**: this study aimed to evaluate the hematological and lymphocyte profile of individuals, as well as identify the ancestral profile through Ancestry Informative Markers (AIMs) from two cities in the Northeast region of Brazil (Salvador-Ba and Feira de Santana-Ba). **Methods**: 58 individuals with COVID-19 classified as mild and severe clinical conditions were analyzed. The lymphocyte profile was identified by immunophenotyping and genotyping by PCR. **Results**: The severe group presented: increased leukocytes and total neutrophils; reduction in the parameters of erythrocytes, hemoglobin, hematocrit and lymphocytes compared to the mild group, as well as a decrease in the cellular profiles of TCD4+, TCD8+ and B lymphocytes. Regarding the ancestral profile, the severe group presented 40.2%, 30.4% and 29.4% of European, African and Amerindian ancestral profiles, respectively. While the light group had 44.8%, 30.9% and 24.3% of European, Amerindian and African profiles, respectively. **Conclusion**: Ancestry was not associated with the severity of COVID-19, but the data here corroborate findings that describe that an individual's immune response plays a crucial role in the pathogenesis of the disease.

Keywords: COVID-19; Ancestry Informative Markers; immune response

Support: FAPEX, FAPESB

IMMUNOREGULATORY POTENTIAL OF CHIMERIC PROTEINS FROM SCHISTOSOMA MANSONI IN HUMAN PERIPHERAL BLOOD CELLS

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Introduction: Allergies are the most prevalent chronic diseases in the world and have been increasing in number of sick people in developing countries, especially in cities from Latin America. Many studies evaluate the effectiveness of immunotherapy with recombinant molecules with the ability to modulate the allergic response. Among the promising molecules, some are present in helminths, since helminth infection activates mechanisms similar to the allergic response, with production of IgE and recruitment of eosinophils, at the same time that seem to favor the polarization of Th1 responses concomitantly with the production of IL-10. **Objectives:** The objective of this work is to test the immunoregulatory capacity of proteins from the Schistosoma mansoni parasite: Elastase Cercarian SmCE in the form of the chimeras SmCET and SmCETB in peripheral blood cells of allergic individuals. **Methods:** Tests were carried out of cytokine measurement in the supernatant of the culture of peripheral blood mononuclear cells from 3 allergic individuals stimulated with different concentrations of recombinant proteins in 24 and 72h. In addition, viability tests are carried out using MTT after the incubation times. **Results and Discussion:** It was observed that stimulation with the chimeras promoted the production of IL-10 and IFN-γ without any stimulation of the generation of IL-5. Th1 profile cytokines such as IFN-γ generate a polarization of the Th2 allergic response, while the regulatory cytokine IL-10 reduces general inflammation caused by allergic conditions. Furthermore, the viability test demonstrated that at low concentrations the proteins can generate cell proliferation after 72 hours of incubation. **Conclusions**: The chimeric proteins seem to be suitable alternatives to allergen-specific immunotherapy. More tests will be carried out with a larger number of individuals to determine the immunoregulatory profile of SmCET and SmCETB.

Keywords: Allergies, chimeric protein, immunomodulation.

Support: FAPESB CAPES

IMMUNOTHERAPEUTIC BTH2 REDUCES EOSINOPHILIC INFLAMMATION IN A MURINE MODEL OF ASTHMA

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Introduction: Allergen immunotherapy (AIT) is an alternative treatment approach with disease-modifying features. Several formulations are currently available in AIT field, including recombinant protein-based technologies. BTH2 is one of such proteins, aiming the tolerance against *Blomia tropicalis* allergy. **Objectives:** The aim of this work was to access the safety and efficacy of BTH2 in a chronic murine model of allergy induced by a mite soluble extract. **Material and Methods:** A/J mice were sensitized by subcutaneous injections of *B. tropicalis* extract (BtE) on days 0, 7, 14 and 21 and by intranasal instillations with BtE on days 27, 29 and 31. Challenges occurred twice weekly for 3 months via intranasal instillations with BtE. Treatment with BTH2 was performed for 3 months, five days a week and using subcutaneous injections, without adjuvants. Bronchoalveolar lavage fluid (BALF) was obtained and, after centrifugation, supernatants were stored at – 20 °C for quantification of cytokines. The cell pellet was used for total cell counts, cytospin for differential counts of leucocytes, and dosage of eosinophils peroxidase (EPO) activity. **Results and Discussion**: Total cell counts revealed that the treatment with BTH2 significantly reduced the number of leucocytes in comparison with placebo group (Sham). Similarly, the number of neutrophils and eosinophils were significantly decreased when compared to Sham. In contrast, the number of lymphocytes were significantly increased upon BTH2 treatment. EPO activity was also significantly reduced in BALF of BTH2-treated mice compared to Sham. All analyzed type 2 cytokines (IL-4, IL-5 and IL-13) were reduced by BTH2 treatment in BALF, but only IL-5 showed significant results compared to Sham. While the innate cytokine IL-1β was significantly reduced, BTH2 group showed significant higher presence of IL-10, IFN-g and TNF in the BALF. **Conclusions:** Considering these results, we concluded that our chronic experimental model can be used for verification of safety and efficacy of an immunotherapeutic. In fact, BTH2 reduced not only the eosinophilic but also the neutrophilic inflammation, seemly inducing a regulatory and/or Th1-biased immune response. However, further analysis on other samples of this murine model will be verified in future assays to confirm our conclusions.

Keywords: allergy; *Blomia tropicalis*; hypoallergen; treatment

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IMPROVEMENT OF THE IDENTIFICATION OF MULTINUCLEATED PLASMA CELLS IN BONE MARROW ASPIRATES SLIDE IMAGES BY ARTIFICIAL INTELLIGENCE

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Introduction: Multiple myeloma (MM) is a hematologic malignancy characterized by the uncontrolled proliferation of plasma cells within the bone marrow, contributing to 1% of all global cancer cases. In addition to a myelogram, a final diagnostic approach includes supplementary assessments such as complete blood counts, biochemical analyses, calcium level measurements, imaging studies, immunophenotyping, and genotyping. The evaluation of plasma cell infiltration through myelogram, which is contingent on an observer's interpretation, proves to be a time-consuming process conducted by hematologists. The I-CARE Project conducted by LABIMUNO and LabIA at UFBA is developing an Artificial Intelligence (AI) to identify and quantify nucleated cells in bone marrow aspirate slide images to improve myelogram diagnosis quality and speed. **Objectives:** The present research aims to improve the identification capability of multinucleated plasma cells in bone marrow aspirate slide images by Artificial Intelligence in development by I-CARE Project. **Methods:** By using Machine Learning with Deep Neural Networks, the project has been training an AI capable of detecting plasma cells in bone marrow slide images. The dataset used for the training was evaluated in two sizes, first with 629 plasma cell images having 32 being multinucleated, and second with 1891 plasma cell images having 36 being multinucleated. The AI was evaluated for its ability to correctly detect multinucleated plasma cells. **Results and Conclusions**: Both AIs analyzed were able to detect plasma cells, and in both the multinucleated plasma cells were detected. However, there was a significant difference in the ability to identify a multinucleated plasma cell as one cell. In the first dataset, it was noticed that a multinucleated plasma cell has been identified as multiple cells, according to the total number of nuclei. The AI from the second dataset was able to correctly identify a multinucleated plasma cell as one cell independently of how many nuclei it presents. The present study was able to improve the identification capabilities of a plasma cell detecting AI through increasing dataset images of the plasma cell and its anomalous characteristics.

Keywords: Multiple Myeloma; Artificial Intelligence; Data Science

Support: CAPES; PIBIC; CNPq; FAPESB; UFBA; LabImuno-UFBA; HUPES; ICS-UFBA; IC-UFBA.

INVESTIGATION INTO THE ROLE OF POLYMORPHISMS IN THE INTERFERON-GAMMA GENE IN DIFFERENTE ASTHMA PHENOTYPES

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Introduction: Asthma is a multidimensional inflammatory disease characterized by the presence of respiratory symptoms that trigger the inflammatory process, which is accompanied by several immune cells and cytokines such as interferon-gamma (IFNG). This cytokine has been associated to immunopathogenesis and different phenotypes of asthma, and polymorphism in the *IFNG* gene has been studied in this context. **Objectives:** To investigate the role of variants in the *IFNG* gene in T2 and non-T2 asthma phenotypes. **Material and Methods:** A total of 601 individuals were selected from the *Program for Asthma Control in Bahia* (ProAR) and stratified into T2 asthma (n = 225, eosinophil data ≥225UI/mL and positive skin prick test for at least one allergen and/or positive IgE above 0.70) and not T2 (n = 376, without previous requirements). The individuals were submitted to previous lung function data evaluation, skin prick test for the most common allergens in our region, genomic DNA extraction for genotyping and IFNG measurement in peripheral blood. In silico analysis were carried out on the available secondary database using PLINK 2.0. The SPSS software was used for normality, variance analysis and chi-square tests as well as logistic regression. **Results and Discussion**: Our preliminary data showed a predominance of females (80.19%), individuals with overweight (67.2%) and non-smokers (68.05%) in our sample. Severe asthma was observed in 47.23% of individuals, with 74.23% showing reversibility to bronchodilator. In genetic analysis, a total of 14 single nucleotide variants (SNVs) of the *IFNG* gene were selected by quality control and 8 showed significant results for asthma severity in the general sample, particularly 2 with negative outcomes (rs2430561 and rs2069718) and 6 with positive outcomes (rs2069723, rs2069719, rs2069717, rs2069715, rs1861494 and rs74099994). Analyses were also performed on reversibility phenotypes with negative (rs2069713 and rs2069718) and positive (rs1861493) outcomes. In the atopic asthma model, a variant with a negative outcome (rs2069713) was also identified. **Conclusions**: Our preliminary results suggest that several *IFNG* SNVs may implicate asthma severity and reversibility in individuals with T2 and non-T2 phenotypes as well as in atopic asthma. Other analyses are underway to check cytokine levels.

Keywords: asthma; IFNG; phenotypes; immunogenetics; polymorphism

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INVESTIGATION OF THE NEUROPROTECTIVE AND ANTI-INFLAMMATORY ACTIVITY OF MARINE SPONGE EXTRACTS

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Introduction: Neurodegenerative diseases involve the gradual loss of neurons, leading to disability and death. Research into new therapies, focused on natural compounds with anti-inflammatory potential in the CNS, such as marine sponges. Preclinical studies indicate anticancer and anti-inflammatory activity in extracts or compounds isolated from various species of sponges. **Objectives:** To evaluate the cytotoxicity and neuroprotective potential of extracts from marine sponge species against in vitro inflammatory damage associated with the modulation of microglia response. **Methods:** PC12 cells were treated with 20 extracts (0.1 to 200 µg/mL) obtained with DCM, EtOAc and MeOH solvents from species of marine sponges of the genera Aplysina, Cladocroce, Condrilla, Callyspongia and Haliclona. Cell viability was determined after 72 hours of treatment using the MTT test. PC12 cells were subjected to inflammatory damage with LPS for 12 hours and treated for 24 hours with A. fulva extract (1.10 µg/mL), or with its purified compound AF-H1 (1.10 µM), and viability cell evaluated by trypan blue and propidium iodide. Microglia from the cerebral cortex of neonatal Wistar rats were treated for 24 h with conditioned medium from PC12 cells under these conditions and the phenotype was assessed by phase contrast, Rosenfeld staining and immunocytochemistry for Iba-1 and CD68. **Results:** Most of the extracts showed toxicity at concentrations of 100 and 200 µg/mL, except for the Ac-EtO extracts which were from [10 µg/mL]. The MeOH extract of A. fulva and AF-H1 were non-toxic. PC12 cells subjected to damage with LPS showed contracted cell bodies, an effect that was not observed in cultures treated with AF-MeOH and AF-H1 extracts at the adopted concentrations. Microglia treated with conditioned medium from PC12 cultures subjected to LPS showed an amoeboid shape; in contrast, microglia subjected to conditioned medium from PC12 cells treated with LPS and AF-MeOH or LPS and AF-H1 showed a more ramified phenotype, similar to that of microglia under control conditions. **Conclusions:** The results obtained demonstrate that extracts from marine sponges and AF-H1 are not toxic. Treatment with these compounds contributed to a possible reversal of the morphology of PC12 cells and microglia when exposed to damage.

Keywords: neurodegenerative diseases, neuroinflammation, marine sponges, neuroprotection, anti-inflammatory

Support: CNPq; CAPES.

LUTEOLIN ATTENUATES IRINOTECAN-INDUCED INTESTINAL MUCOSITIS IN THE DUODENUM AND COLON OF MICE

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Introduction: Mucositis is a complex inflammatory reaction that occurs in the gastrointestinal tract of patients undergoing chemotherapy or radiotherapy. Currently, there is no fully effective treatment for this condition. Recent studies have explored flavonoids, substances found in medicinal plants, as a possible solution to this problem due to their potential anti-inflammatory and antioxidant properties. Among them, luteolin has antioxidant and anti-inflammatory properties that can prevent the cytotoxic effects of chemotherapy. In addition, the anti-inflammatory effect of luteolin on DSS-induced colitis was demonstrated in vivo. Thus, we hypothesize that luteolin may modulate the inflammatory response and prevent inflammatory parameters, including intestinal histoarchitecture and the distribution of goblet cells and collagen fibers, from being altered in the mucosa of the duodenum and colon. **Objective**: To evaluate the effects of the flavonoid luteolin on inflammatory parameters in the mucosa of the duodenum and colon of mice. **Material and methods**: Eighteen mice were divided into three groups: Naïve, vehicle (Vei, 10 mL/kg water) and luteolin (Lut, 30 mg/kg). After 7 days of treatment, Vei and Lut received intraperitoneal irinotecan (75 mg/kg/day) for 4 days. Euthanasia was performed 72 h after the last dose of irinotecan. Duodenal and colon samples were examined histologically. **Results and conclusions**: luteolin treatment reduced irinotecan-induced histomorphometric changes in the duodenum and colon, prevented goblet cell depletion in the duodenum, and increased type III collagen deposition in the colon. On the other hand, the distribution of type I collagen fibers in the duodenum and colon was reduced. This suggests that extracellular matrix remodeling, characterized by the conversion of collagen III fibers to type I, was modulated by luteolin, as the increase in type I fibers is accompanied by an increase in fibrosis. Our results suggest that luteolin has a protective effect on the intestinal mucosa during treatment with irinotecan.

Keywords: chemotherapy; flavonoids; intestinal histoarchitecture; goblet cells; collagen.

Support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

MTOR GENE VARIANTS ARE ASSOCIATED WITH SEVERE COVID-19 OUTCOMES: A MULTICENTER STUDY.

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Introduction: The worst outcomes linked to SARS-CoV-2 infection have been attributed to the cytokine storm, which appears to be a key player in the immunopathogenesis of the disease. The poor prognosis is also associated with advanced age and pre-existing comorbidities, like diabetes and cardiovascular diseases. The mTORC1 pathway has been proposed as a typical molecular pathway that may be overactivated in people with a genetic predisposition. **Objectives:** In this study, we aimed to investigate the association between *MTOR* gene variants and COVID-19 outcomes. **Material and Methods:** This case-control study recruited mild and severe COVID-19 individuals from Brazilian states. *MTOR* variants were genotyped. Logistic regression analysis and Kaplan-Meier survival curves were performed to explore the clinical significance of the genotypes. We applied a genotype risk score to estimate the cumulative contribution of the risk alleles. TNF and IL-6 plasma levels were measured. **Results and Discussion:** The T allele of the *MTOR* rs1057079 variant was associated with a higher chance of developing the most severe form of COVID-19, and a higher likelihood of need the Intensive Care Unit. In addition, higher levels of IL-6 and COVID-19 death was linked to the T allele of the rs2536 variant. **Conclusions:** These variants showed a cumulative risk when inherited collectively and may be useful in predicting a severe outcome of COVID-19, resulting in a more effective allocation of health resources.

Keywords: mTOR; genetic variants; COVID-19; Severe.

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POLYMORPHISM IN THE TMPRSS2 GENE IS ASSOCIATED WITH ASTHMA CONTROL IN PATIENTS WITH ATTACKS

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Introduction: Asthma exacerbations are episodes characterized by a progressive increase in symptoms of shortness of breath, coughing, wheezing or chest tightness. The disease is often accompanied by a gradual reduction in lung function, which often requires a change in treatment and these episodes can be caused by respiratory viruses. Although many studies show that asthma is not a risk factor for COVID-19, the SARS-CoV virus can trigger exacerbations in people with asthma. In this context, the transmembrane serine protease 2 encoded by the *TMPRSS2* gene in humans is involved in the fusion of the virus with the host cell. **Objectives:** The objective of this study was to evaluate polymorphisms in genes related to SARS-CoV-2 infection in humans, including the *TMPRSS2*. **Material and Methods:** Whole blood was collected from 308 patients who had at least one asthma exacerbation (attack) in the last 15 days and were treated at an Emergency Care Unit (UPA) in Salvador, BA. Genomic DNA was extracted using the FlexiGene kit (Qiagen). Genotyping was performed by RT-PCR using TaqMan probes (Thermo Fisher). The blood count was performed by an automated method using the CELL-DYN Ruby equipment (Abbot). **Results and Discussion:** The rs12329760 in the *TMPRSS2* gene is a missense change that exchanges a valine (Val) for a methionine (Met) at position 160 of the protein. This change is classified as harmful or deleterious. In our study, the T allele of this SNP showed a negative association with lack of asthma control in both an additive model (OR: 0.61; p = 0.029) and a recessive model (OR: 0.27; p = 0.008). Furthermore, in literature this allele is related to a decrease in monocytes in the blood of individuals with the TT genotype. **Conclusions:** Therefore, the present study demonstrates that there is probably a protective relationship between polymorphisms in genes related to SARS-CoV-2 infection in people with asthma attacks.

Keywords: asthma, attack, *TMPRSS2*, COVID-19, polymorphism

Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação ProAR and National Institute for Health Research (NIHR)

POLYMORPHISMS ON *IFNA* GENE LINKED WITH NON ATOPIC ASTHMA ON CHILDRENS

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Introduction: Variations in the human genome can trigger the most diverse types of alterations in the phenotypes expressed by individuals. Single Nucleotide Variant (SNV) polymorphisms may be responsible for these subtle variations in a wide variety of known genes. Environmental factors may also be involved in the most varied forms of pathology presented. Non-atopic asthma fits the profile of asthma as a T2-low response, where there is no need for IgE involvement, which characterizes a response to some type of allergen, so this study attempts to propose, through the investigation of variants, genetic profiles associated with non-atopic asthma using a large cohort of children from a large urban center in Salvador, Bahia, Brazil. **Objectives:** Our objective is to evaluate whether polymorphisms in the gene encoding IFN-α are associated with non-atopic asthma in children and their possible effects on the phenotype in question. **Methods:** The study included 1246 individuals from the SCAALA program aged between 4 and 11 years. The DNA of these children was extracted from peripheral blood and 4 variants were subsequently genotyped using the 2.5 HumanOmni Beadchip from Illumina (San Diego, California, USA). The results found described SNV associations with skin prick tests and IgE dosage and pre-defined groups (atopic asthmatics, non-atopic asthmatics, atopic non-asthmatics and non-atopic non-asthmatics). **Results and Conclusions** The T allele of the rs34244195 variant was positively associated with non-atopic asthmatics compared to the atopic control group (OR:1.7, 95% CI 1.10 – 2.61) and the G allele of the 10120977 variant was positively associated with the presence of total IgE (OR:1.4 CI95% 1.0 – 1.9 P:0.01) and positive skin test for at least one allergen (OR: 1.0 CI95% 0.67 – 1.7 P:0 .04), no variants were found significantly associated with the atopic asthma phenotype. Polymorphisms in the IFN-α gene were correlated with the non-atopic asthma phenotype and the presence of atopy in our population. The impacts of these variants on the phenotypes presented here need to be the subject of further analysis using other types of population to fill other gaps in the literature.

Keywords: Asthma; Single Nucleotide Variant; Non atopic Asthma; IFNA.

Support: CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

PRODUCTION AND EVALUATION OF NEW CHIMERIC ANTIGENS TO TOXOCARA SPP. IMMUNODIAGNOSIS OPTIMIZATION

Raphael Chagas Silva, Vitor Lima Miranda Melo, Eduardo Santos da Silva, Carolina Orrico Melo Ferreira de Jesus, Antônio Márcio Santana Fernandes, Álvaro Augusto Cruz, Natália Gomes de Morais Coneglian, Neuza Maria A. Neves, Carina da Silva Pinheiro.

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Introduction: Human toxocariasis is a worldwide neglected disease caused by species of Toxocara gender. The disease is developed during the third stage larvae migration through the host body activating the immune system trigger with inflammatory responses Th2-drived. The infection diagnosis is performed using a gold standard secreted-excreted of Toxocara spp. antigens but the existent antibodies cross-reactivity, variation of sensibility and specificity values due to non-reproducible methodologies render this test imprecise for diagnosis. Our research group has previously utilized two highly sensitive and specific recombinant proteins from the Toxocara proteome as an alternative diagnostic approach to mitigate TES-ELISA inconsistencies. **Objectives**: In this work, we developed and tested two purified chimeric proteins (rSHORT and rFULL) on toxocariasis immunodiagnostic performances by indirect ELISA. **Material and Methods**: We performed Dot Blot for reactivity evaluation and comparative indirect ELISA using new chimeric proteins and a mix of two recombinant proteins previously studied as gold standard in 232 sera samples from the Brazilian Program of Rhinitis and Asthma Control. **Results**: Our findings demonstrated that the rSHORT protein exhibited comparable reactivity in sera compared to the Dot Blot protein mixture, while the rFULL protein displayed lower reactivity and lower similarity with the gold standard used. The rSHORT protein yield IgG and IgG4 sensitivities and specificities higher than rFULL, in both adsorbed and non-adsorbed sera (rSHORT and rFULL IgG sensitivities: 98.6% and 93.7%; IgG4: 96.5% and 88%, in absorbed sera; rSHORT and rFULL IgG specificities: 94.4 % and 86.7%; IgG4: 92.2% and 87.8% in absorbed sera. In non-absorbed sera, the validation parameters showed better results for sensibilities and specificities (rSHORT and rFULL IgG sensitivities: 99.3% and 89.7%; IgG4: 98.5% and 89.4%; rSHORT and rFULL IgG specificities: 97.8 % and 81.1%; IgG4: 97.7% and 87.8%). **Conclusion**: Given the higher signal of rSHORT protein and based on the similarity of results reached for both gold standard and rSHORT proteins, the chimeric rSHORT antigen represents a strong candidate to toxocariasis immunodiagnostic optimization.

Keywords: Toxocara; Immunodiagnostic; Chimeras; Antibodies.

Support: CNPQ UNIVERSAL: 403336/2021-0, CAPES, and FAPESB are also acknowledged for funding this project.

PRODUCTION OF *SCHISTOSOMA MANSONI* CHIMERIC ELASTASE PROTEIN

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Introduction: The prevalence of chronic allergic diseases has been increasing in the global population. Current treatments aim to manage symptoms, as no known cure is available. The most effective treatments involve corticosteroids, but their use is associated with several side effects that can impact the patient's well-being. The immune response triggered by allergens is similar to the immune response against helminths, in which parasite antigens stimulate $CD4^+$ T lymphocytes to produce Th2-type cytokines such as IL-4 and IL-5. Specifically, in response against *Schistosoma mansoni*, IL-10 is also produced in order to control the immune response to the parasite. During schistosome infection through cercariae penetration in the host skin, certain proteins make initial contact with the human body and trigger immediate evasion strategies employed by the parasites. Among these proteins, elastase is the most abundant protein in cercaria secretions, and previous studies demonstrate that this protein is capable of inducing a specific immune response in the host. **Objectives:** Our work aims to produce a chimeric elastase protein (*Sm*CETB) in order to evaluate its effect on allergy modulation and treatment. **Methods:** A synthetic plasmid containing *Sm*CETB sequence was transformed into different strains of *E. coli* and heterelogous protein expression was induced with IPTG for 4 and 24 hours. Bacterial extracts were solubilized in Tris HCl pH 9.5. Purification was carried out by affinity chromatography. Protein expression and purification were confirmed through Western Blot (WB). **Results and Conclusions:** The chimeric protein *Sm*CETB was designed using predicted B and T epitopes from schistosome elastase, resulting in a 24kDa-sized protein. The synthetic plasmid was successfully transformed in both Rosetta and Star bacterial strains. Additionally, *Sm*CETB protein expression was observed and confirmed by WB after both 4 and 24 hours of expression in the bacterial culture. Affinity purification experiments were also conducted using the histag inserted in the protein's C-terminal end. With *Sm*CETB successfully expressed and purified, our group will be able to conduct future experiments testing human peripheral blood cells *in vitro* and also test *Sm*CETB in both acute and chronic murine allergy models.

Keywords: Cercariae, allergies, chimeric protein, purification, immunomodulation.

Support: PIBIC – UFBA

PRODUCTION OF *SCHISTOSOMA MANSONI* CHIMERIC PROTEIN CONTAINING T CELLS EPITOPES FROM ELASTASE

Emilly de Jesus Araújo Santos[1], João Vitor Borges Rios[1], Raphael Chagas Silva[1], Eduardo Santos da Silva[1], Carina da Silva Pinheiro[1], Carolina Orrico Melo Ferreira de Jesus[1], Bárbara Castro-Pimentel Figueiredo[1],[2]

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Introduction: Allergies are the most prevalent chronic diseases worldwide, and the number of allergic patients in developing countries is increasing. Currently, there is no curative treatment available. Among the palliative medications, corticosteroids are the most effective, but they come with serious side effects when used over the medium and long term. It is known that helminth infection, especially its antigens and immune evasion mechanisms, stimulate a regulatory reaction resulting in the production of IL-10. This phenomenon is beginning to be associated in the literature with allergy control induced by the parasite, as it modulates the Th2 response. During *Schistosoma mansoni* infection through cercariae penetration in the host skin, certain proteins make initial contact with the human body. Among these proteins, elastase is the most abundant protein in cercaria secretions, and previous studies demonstrate that this protein is capable of inducing a specific immune response in the host. **Objectives:** Our work aims to produce a chimeric elastase protein (*Sm*CET) in order to evaluate its effect on allergy modulation and treatment. **Methods:** A synthetic plasmid containing *Sm*CET sequence was transformed into different strains of *E. coli* and heterelogous protein expression was induced with IPTG for 4 and 24 hours. Bacterial extracts were solubilized in Tris HCl pH 9.5. Purification was carried out by affinity chromatography. Protein expression and purification were confirmed through Western Blot (WB). **Results and Conclusions:** The chimeric protein *Sm*CET was designed using predicted T epitopes from schistosome elastase, resulting in a 11kDa-sized protein. The synthetic plasmid was successfully transformed in both Rosetta and Star bacterial strains. Additionally, *Sm*CET protein expression was observed and confirmed by WB after both 4 and 24 hours of expression in the bacterial culture. Affinity purification experiments were also conducted using the histag inserted in the protein's C-terminal end. With *Sm*CET successfully expressed and purified, our group will be able to conduct future experiments testing human peripheral blood cells *in vitro* and also test *Sm*CET in both acute and chronic murine allergy models.

Keywords: Cercariae, allergies, chimeric proteins, immunomodulation.

Support: PIBIC-UFBA

PROFILE AND PROGNOSTIC VALUE OF LYMPHOCYTE SUBSETS IN PATIENTS WITH MULTIPLE MYELOMA UNDERGOING THERAPY WITH CYCLOPHOSPHAMIDE, THALIDOMIDE, DEXAMETHASONE AND DARATUMUMAB

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Introduction: Multiple Myeloma (MM) is the second most common hematological malignancy, resulting from the proliferation of monoclonal protein-producing plasma cells, predominantly affecting the elderly population. In the last decade, therapeutic advances have led to an increase in the overall survival of patients, however the disease remains incurable. Therapeutic protocols combining alkylating agents, immunomodulators, immunosuppressants, and immunotherapy induce an immunological shift that is still not fully understood. **Objectives:** The aim of this study was to characterize lymphocyte subsets in patients with Multiple Myeloma eligible for Autologous Stem Cell Transplant (ASCT) using first-line therapy with cyclophosphamide, thalidomide, dexamethasone combined with daratumumab (CTd-Dara), an anti-CD38 monoclonal antibody. **Methods:** Between 2018 and 2022, 23 newly-diagnosed MM patients had their lymphocyte profiles analyzed at five distinct time points, and the therapeutic response was monitored by Next-Generation Flow (NGF), through the detection of measurable residual disease (MRD). The mean fluorescence intensity of the CD38 molecule on the surface of plasma cells was evaluated by flow cytometry as a prognostic tool. **Results and Discussion:** It was observed that the treatment induced significant changes in the lymphocyte profile, with emphasis on the decrease in B cells and NK cells. The composition of the B cell subsets changed significantly throughout the treatment. When evaluating prognostic variables, the expression of the CD38 molecule on the surface of plasma cells emerged as a promising marker, correlating with lower MRD levels for this therapy and the R-ISS system. Although the lymphocyte subpopulations and Circulating Tumor Cells (CTCs) did not achieve statistical significance in prognostic terms, they indicate a pattern warranting further investigation. **Conclusion:** These findings enhance our understanding of the immunomodulatory effects of therapies in MM and signal ways to optimize treatments and patient monitoring.

Keywords: Multiple Myeloma; Daratumumab; Lymphocytes subsets; CTD; Plasma Cells.

Support: Labimuno; Janssen.

PROTECTIVE EFFECTS OF NICOTINE AGAINST AMINOCHROME AND ALPHA-SYNUCLEIN-INDUCED STRESS IN ASTROCYTES

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Introduction: Alpha-synuclein has been identifying as the main proteinaceous aggregate in Lewy bodies, which contribute to progressive neuronal death in Parkinson's disease. The role of astrocytes in Parkinson's disease (PD) pathogenesis is not fully clear. Some pre-clinical studies have shown their involvement in neuroinflammation, on the other hand, aminochrome, an ortho-quinone formed during dopamine oxidation to neuromelanin, is able to induce astrocyte death, and alpha-synuclein immunoreactive astrocytes are present in post-mortem PD patients' samples. PD is still a disease without a cure and the search for neuroprotective compounds is current. Nicotine, the main compound from Nicotiana tabacum, is able to protect neurons against aminochrome toxicity; however, the effect in dysfunctional astrocytes was not investigated. Objectives: In this study, we evaluated the effect of nicotine on glial cells expressing human alpha-synuclein and/ or treated with aminochrome. Methodology: We used primary culture of mesencephalic cells from rat embryos (E15) and Wild-type U251 human astrocytes or U251 transfected for A53T alpha-synuclein – nYFP super-expression. These cells were treated with nicotine and/ or aminochrome. The cell viability was assessed by MTT test or propidium iodide test, cell morphology was accessed by Rosenfeld's staining, and analysis of autophagy dysfunction was performed by Western Blotting for LC3 and P62 astrocytes from cell lineage or submitted to treatment with nicotine and/ or aminochrome. The MTT test was performed to assess the cell viability. Results: We observed that nicotine induces vacuolation and protects cells in primary cultures against aminochrome neurotoxicity. We also observed that 10 µM nicotine for 6 h increased the expression of LC3II in wild-type U251 cells. Moreover, nicotine (0.1 – 50 µM, for 48 and 72h) protects transfected alpha-synuclein U251 cells against aminochrome cytotoxicity. Conclusion: Nicotine is protective against aminochrome and alfa-synuclein astrocytic damage in astrocytes. These results reinforce the potential application of nicotine in PD therapy.

Keywords: Parkinson's disease; astrocytes; nicotine; neuroprotection

Support: CNPq; Fapesb, CAPES

SEROPREVALENCE OF SARS-COV-2 VIRUS INFECTION IN BLOOD DONORS IN THE INTERIOR OF BAHIA

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Introduction: COVID-19 brought complications to several countries. In Brazil, the distribution of the infection occurred, in general, from the state capitals to the inland cities. This flow was also identified in Bahia, and it is important to trace the level of viral circulation and the impact of infection in the regions of the state. **Objective:** To investigate the relationship between seroprevalence for SARS-CoV-2 infection in blood donor samples and the notification of cases of virus infection in the interior of Bahia in 2020. **Material and Methods:** The population sample originally consisted of 6,877 blood donors in 13 cities located in four regions of the state of Bahia (north, south, east, and west). Subsequently, the serum samples were tested for the detection of anti-SARS-CoV-2 antibodies, using the TR COVID-19 IGM/IGG Kit – Bio-Manguinhos. Statistical analyses were conducted to estimate the crude seroprevalence of the infection, and the Bayesian correction method was used to determine the seroprevalence adjusted by the state's region. The data related to notification/ incidence are sourced from the public database of the State Health Department of Bahia (SESAB) and DATASUS. Only records where the test for Covid-19 was marked as "POSITIVE" and "SARS related to COVID-19" in the year 2020, between the months of April and November, were retained. Results: Blood donors had a median age of 34 years (IQR: 25-42). Most of the donors tested were from the western region of Bahia. The seroprevalence among blood donors and the cumulative incidence of infection/hospitalization between April and November 2020 were 12,2% and 5.0%, respectively. It was possible to identify differences between seroprevalence in blood donors and cumulative incidence of cases, mainly in the western and eastern regions of Bahia, in November 2020. **Conclusion:** Identification of underreporting of SARS-CoV-2 infection and the knowledge about the distribution of the infection in the state of Bahia may contribute to the development of more effective public strategies to control the spread of SARS-CoV-2.

Keywords: COVID-19; SARS-CoV-2; Seroprevalence; Bahia; Underreporting. **Support:** Fiocruz Support Foundation (FIOTEC). INOVA/FIOCRUZ Program (2020). Process Number: 48401626961258. CAPES.

STUDY OF PATHOLOGICAL ALTERATIONS IN THE CEREBELLUM OF RODENTS IN THE PARKINSON DISEASE STUDY MODEL INDUCED BY AMINOCHROME

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Introduction: Parkinson's disease (PD) is an age-predictive neurodegeneration, characterized by the loss of neurons due to metabolic disturbances or molecular damage induced by dopamine metabolites, involving mitochondrial dysfunction, accumulation of protein clusters, oxidative damage, and inflammation. **Objectives:** The objective of the study was to analyze the cerebellar morphological alterations in neurodegeneration and the prophylactic effect of rutin in a preclinical model of PD. **Methods:** The damage was induced by stereotaxis and injection of 6 nM aminochrome into the striatum of Wistar rats (8-10 weeks of age). After stereotaxis, an animal group received oral supplementation with rutin 10 mg/kg, for 21 days. The cerebellar region was analyzed by histochemistry (hematoxylin/eosin and cresyl violet). **Results and Conclusions:** Aminochrome induces perivascular and perineuronal edema, chromatolysis, and neuronal cytoplasmic vacuoles in the region of the cerebellar peduncle and brainstem of rats. No morphological changes were observed in the cerebellar strata of animals exposed to aminochrome but, significant decrease ($p=0.034$) in Nissl substances in Purkinje, when compared to the control. Rutin protects neurons against damage induced by aminochrome, however, the presence of perivascular and perineural edema was observed in the same fields of histological images. In conclusion, aminochrome induces neural disorders, characterized by structural changes in cells of the cerebellar peduncle and brainstem, leading to decreased protein synthesis in Purkinje cells. Damage in peri-cerebellar regions may be a starting point in signaling cascades for protein dysfunction in Purkinje cells, contributing to the first clinical sign of PD, even with preservation of cerebellar cellular architecture.

Keywords: neurodegeneration, Parkinson's disease, rutin; aminochrome; cerebellum.

Support: FAPESB.

SUMMARY OF DATA AND PRODUCTS OF THE THESIS ENTITLED ANTIGENIC REACTIVITY OF RECOMBINANT PROTEINS RSPAC, RPKNG, RNANH E RSODC DERIVED FROM *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* WITH HUMAN, GOAT AND OVINE SERA.

Ramon Mendes dos Santos, Caio Lopes Borges Andrade, Gabriel Saldanha Müller, Maria Izabel Cerqueira, Izabela Maria Del Rei, Silvana Marchioro, Luan Santana Moreira, Rogério Reis Conceição, Marcos Borges Ribeiro, Nubia Seyffert, Maria Isabel Schinoni, Eduardo Martins Netto, Roberto Meyer, Songelí Menezes Freire

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Introduction: *Corynebacterium pseudotuberculosis* is one of the etiologic agents causing lymphadenitis or pneumonia in humans, and caseous lymphadenitis in goats and sheep. The current serological diagnosis for animals has a protocol of low specificity and sensitivity, and in humans there is no serological diagnostic kit. **Objectives:** In this work, we intended to evaluate the antigenic potential of four recombinant proteins of *C. pseudotuberculosis* (rSodC, rPknG, rNanH and rSpaC) in goat and sheep sera and two of them (rPknG and rSodC) in human serum. **Methods and conclusions:** In the animal model, goats and sheep were experimentally infected with inoculums of CAP76, CAP21 or VD57 strains (n=16), and another group of these animals was not infected (n=12). In the human model, serum was obtained from two groups: Group 1: participants who care for goats and sheep (n=14), and Group 2: participants who reported not having contact with these animals (n=25). Recombinant proteins were produced in *E. Coli* BL21 (DE3) Star, and antigenicity was evaluated using ELISA and/or Western blot techniques. Homology analyzes and epitope prediction of rPknG and rSodC were performed *in silico*. With the results obtained, it can be suggested that rSodC is a strong candidate as a tool for the diagnosis of infection by *C. pseudotuberculosis* in goats and sheep. In the human model, rPknG and rSodC reacted indiscriminately, with no difference between the evaluated groups. It was observed, in the *in silico* analyses, that rPknG and rSodC have high homology coverage with other species of the *Corynebacterium*, *Mycobacterium*, *Rhodococcus*, *Nocardia* (CMRN) group. **Final comments:** Doctoral student received a CAPES CNPq scholarship. During the development of this doctoral thesis project, several actions were developed, in addition to the generation of results and publications. Throughout the time of this doctorate at PPGIm, authors participated in UFBA seminars, UFBA congress, SBI congress, doctoral student tutored 2 Scientific Initiation students at PIBIC UFBA, mentored 1 biomedicine student, 1 course completion monograph of the Biotechnology course, 1 article published in the magazine (doi.org/10.1007/s00284-022-02974-7) and submission of 1 article in the The Brazilian Journal of Infectious Diseases).

Keywords: *Corynebacterium pseudotuberculosis*, human diagnosis, protein kinase G (PknG), superoxide dismutase C (SodC), recombinant protein.

Support: Fundação de Apoio à Pesquisa e à Extensão (FAPEX)

THE RS11385942257 VARIANT IS ASSOCIATED WITH THE RISK OF ICU ADMISSION AND SHORTER SURVIVAL IN COVID-19 PATIENTS

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Introduction: COVID-19 is an infectious disease caused by SARS-COV-2 that has led to thousands of deaths worldwide. Symptoms can vary between individuals, however, in severe cases, they can progress to pneumonia and a systemic inflammation called cytokine storm. It is suggested that the genetics of the host are a factor in this symptomatic heterogeneity since certain genes can influence the outcome of the disease. The LZTFL1 gene is located on chromosome 9, in a region strongly associated with the severity of COVID-19. **Objectives:** Thus, this study aims to investigate in the host the rs11385942257 variant of the LZTFL1 gene linked to the severity of COVID-19. **Methods:** Our study population includes 290 cases from collaborations in different regions of Brazil. Genomic DNA was used to genotype the rs11385942257 variant via RT-PCR. Serum levels of IL-6 and TNF were measured by enzyme immunoassay. The results were analyzed in association with severity, admission to intensive care units (ICUs), and death by logistic regression using PLINK 1.9. In comparison with genotypes, cytokines were analyzed in GraphPad Prisma using the Kolmogorov-Smirnov tests, followed by Kruskal-Wallis or Mann Whitney. **Results and Conclusions:** Our results revealed a positive association of rs11385942257 with the risk of ICU hospitalization in the additive (OR=1.95, CI=1.03-3.68, p=0.03), dominant (OR=2.08, CI=1.07-4.01, p=0.02) and heterozygous (OR=2.14, CI=1.11-4.15, p=0.02) models. No associations were observed with severity and death. However, the survival analysis showed a reduced risk of death over a short period for the AA genotype compared to the other two groups (p=0.004). No relationships were observed between IL-6 and TNF cytokine levels. Our findings suggest that rs11385942257 present in the LZTFL1 gene is associated with an increased risk of COVID-19 progression, leading to the need for ICU admission.

Keywords: COVID-19, LZTFL1, severity, genetic variant, polymorphism.

Support: CAPES, PPSSUS.

USE OF BIOINFORMATICS TOOLS TO PREDICT EPITOPES CAPABLE OF DETECTING SARS-COV-2 IN THE POPULATION OF BAHIA

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Introduction: The global outbreak of COVID-19 triggered by SARS-CoV-2 has highlighted the urgent need to identify molecules that can be used in the development of treatments and vaccines. The search for peptide sequences capable of specifically interacting with the virus is a promising strategy. **Objectives:** Identify in silico peptide sequences with potential to bind to SARS-CoV-2, using bioinformatics approaches and molecular modeling techniques. **Methods:** The first genomic sequences identified in Brazil were subjected to alignment with other betacoronavirus sequences using the MAFFT tool. SARS-CoV-2 protein sequences were obtained from UniProt (taxonomy_id:2697049) for the prediction of T and B cell peptides that can be recognized by the class I histocompatibility system (MHC) with alleles from the Bahia population. For molecular dynamics, protein models were obtained from the RCSB PDB. Molecular dynamics simulations were performed to study the interactions between the selected peptide sequences and the viral protein. To carry out molecular docking, the PyMOL 2.0 software was used to identify the most likely interaction positions between the peptides and the viral protein. The interactions between the selected peptides and the virus protein were investigated through molecular dynamics simulations using GROMACS 5. **Results and conclusion:** The use of bioinformatics in this approach allowed the identification of the various energy levels and which is best suited for binding peptides to protein models, a potential for recognizing SARS-CoV-2 by MHC class I. 26 B cell peptides were also predicted, of which 7 are fundamental proteins in the infectious process. Analysis of molecular dynamics simulations and molecular docking showed significant interactions between these peptides and the viral protein, suggesting a potential for binding and, possibly, use in a therapeutic approach. The study also demonstrates the potential and significance of using bioinformatics and molecular modeling in identifying sequences with potential for the recognition of SARS-CoV-2 and use in COVID-19 therapeutics.

Keywords: SARS-CoV-2 , Bioinformatics, Epitopes, Allelles MHC.

Support: Capes, Fapex, Fapesb

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SUMÁRIO

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POSTER ABSTRACT

CARNOSINE ALTERS ASTROCYTE MORPHOLOGY AND REACTIVITY MARKERS

Parmeggiani, B.[1,2]; Diniz, F.C.[1,2,3]; Brandão, G.[1]; Teixeira M.F.[1,2]; Reis, R.A.M.[2]; Schuck, P.F.[1]; Ferreira, G.C.[1,2]

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Introduction: Carnosine is an imidazolic dipeptide found in several mammalian tissues, including the brain. It has been described as an antioxidant, metal chelator and anti-tumor molecule (amongst other roles), being considered as a potential neuroprotective agent. However, children affected by carnosinemia, an inborn error characterized by accumulation of carnosine, present with severe neurological symptoms. **Objectives:** Contribute to the elucidate the effects of carnosine *per se* in the brain, by investigating if/how important astrocytic markers change after exposure to carnosine. **Material and Methods:** Primary astrocyte cultures were obtained from cerebral cortex of newborn Wistar rats. Cells were kept in DMEM-F12 containing 10% fetal bovine serum, which was renewed every 48h. After a purification step, primary astrocytes were incubated with 0.1, 1 or 5mM of carnosine for 72h. Then, cells were either fixed for immunocytochemistry or scraped in RIPA buffer for western blotting analyses. **Results:** At lower concentrations (0.1 and 1mM), carnosine had no apparent effect on astrocyte morphology when stained for glial fibrillary acidic protein (GFAP), whereas 5mM carnosine induced morphological alterations strongly suggestive of astrocytic reactivity. The immunocontent of GFAP itself was increased by carnosine (5mM) as well. Meanwhile, we found a decrease in the content of glutamine synthetase (GS), and decreased GS activity, without altering aldehyde dehydrogenase 1 family member L1 (ALDH1L1) content. This not only indicates that astrocytic function might be compromised in the presence of this dipeptide, but also that carnosine seems to induce astrocytic reactivity with a particular signature of alterations. **Conclusions:** These data imply that carnosine, an otherwise considered as a purely beneficial nutraceutical, causes specific astrocytic alterations, which could impair neurological function in a physiological context, and that its accumulation might also be implicated in the pathophysiology of carnosinemia.

Support: FAPERJ (#201.888/2020), CNPq, CAPES

Keywords: astrocytic reactivity; brain development; L-histidine-containing dipeptide; glial marker.

GALACTOSE IMPAIRS MOTOR FUNCTIONS ONLY IN MALE YOUNG RATS: INVOLVEMENT OF DOPAMINE

Bruna Klippel Ferreira[1]; Thiago Paz Simões[2]; Patricia R. F. Gonçalves[2]; Gilda Angela Neves[2]; Ricardo Augusto de Melo Reis[3]; Patrícia Fernanda Schuck[1]; Gustavo da Costa Ferreira[1]

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Introduction: Galactosemias are inherited diseases that occur due to failure in genes encoding Leloir´s pathway enzymes. Galactosemic patients present symptoms related to cerebellar damage. These symptoms may be related to the time of exposure to galactose in the neonatal period. **Objectives:** Investigate the motor performance and dopaminergic system in cerebellum after acute Gal administration. **Material and Methods**: Thirty-day-old male and female Wistar rats were used. The animals were randomized into the following groups: I) Gal group, which received a single subcutaneous administration of Gal (5 µmol/g of body weight); II) control group, which received the vehicle solution under the same conditions. One, 3 or 24 hours after administration, the animals were evaluated in the rota-rod test. **Results:** Motor performance was impaired in males (but not in females) 3 hours after Gal administration. Then, the cerebellar hemisphere and vermis were extracted for biochemical examinations at this time point (3 hours after Gal administration). Lower content of TrkB-FL, p-CREB, TH and GAD was observed in cerebral hemispheres of males. No differences were detected on the D1R levels or on MAO activity. In contrast to hemispheres, TH content was higher in cerebellar vermis of males receiving galactose, without changes in p-CREB content. Females did not show changes in TH, p-CREB or GAD immunocontent neither in cerebellar *vermis* nor in hemispheres. **Conclusions**: These results demonstrate that the motor impairment induced by Gal depends on the time of exposure. In addition, males are more susceptible to Gal toxicity. Thus, the cerebellum and the dopaminergic system may play an important role in the pathophysiology of galactosemias. Therefore, the investigation of the cerebellar mechanisms involved in the pathophysiology of motor damage may lead to new therapeutic strategies and assure a better quality of life for galactosemic patients.

Keywords: cerebellum, dopamine, galactosemia.

Acknowledgments: CNPq, CAPES, FAPERJ

EFFECT OF ACUTE HYPERPHENYLALANINEMIA ON CHOLINERGIC PARAMETERS IN BRAIN RAT

Santos-Correa, I.S.[1]; Rodrigues, M.T.[1]; Gomes, M.L.[2]; Kist, L.W.[3]; Bogo, M.R.[3]; Streck, E.L.[2]; Zugno, A.I.[2]; Ferreira, G.C.[1]; Schuck, P.F.[1]

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Introduction: Phenylketonuria (PKU) is an inborn error of phenylalanine (Phe) metabolism, caused by a deficiency of the enzyme phenylalanine hydroxylase. It leads to the accumulation of Phe in tissue and body fluids of patients (hyperphenylalaninemia; HPA). Brain injury is the main clinical finding of PKU patients. However, the pathophysiology of cerebral damage is poorly understood. **Objective:** To investigate the effects of HPA on parameters of cholinergic neurotransmission in rat brain. **Materials and methods**: For the *in vivo* experiments, animals received a single subcutaneous administration of 0.9% saline (control group) or 5.2 µmol/g Phe plus 0.9 µmol/g p-chlorophenylalanine (HPA group). One hour after the administration, the animals were euthanized by decapitation; brain structures were isolated and homogenized in specific buffers. ChAT and AChE activities were determined spectrophotometrically. mRNA content was measured by RT-PCR. Determination of Phe and acetylcholine (ACh) levels was performed using commercial kits. **Results**: Results were considered statistically significant when $p<0.05$. AChE activity was higher in the striatum of HPA animals in comparison to the control group. Moreover, ACh levels were significantly lower in all the brain structures of HPA animals. On the other hand, AChE mRNA content and ChAT activity were not altered by HPA. **Conclusion**: Our results suggest that HPA induces cholinergic alterations. Since cholinergic imbalance is associated with neurological failure and progressive decline in learning and memory functions, it is tempting to speculate that these mechanisms may contribute to the intellectual disability observed in PKU patients.

Financial support: CAPES, CNPq and FAPERJ.

Keywords: Acetylcholinesterase, brain, phenylketonuria.

THE USE OF ESCITALOPRAM AND FLUOXETINE IN THE COVID-19 PANDEMIC: A COMPARATIVE ANALYSIS

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INTRODUCTION: The pandemic caused by SARS-CoV-2, which began in December 2019, was a milestone in the lives of many people around the world. A study carried out in Turkey identified higher scores for anxiety and depression in individuals: females; urban area residents; with relatives and friends with COVID; with a history of previous or current psychiatric illnesses. In Brazil, between 2019 and 2020, there was a 13.84% growth in the sale of antidepressants, namely escitalopram and fluoxetine, which had bioavailability of 80% and 90% respectively. **METHODOLOGY:** A systematic search was carried out for articles that met the following inclusion criteria: Articles published between 2019 and 2021; Have experienced the COVID-19 pandemic; Articles carried out on humans; Whether or not they practice physical activities; Be between 18 and 29 years old. **RESULTS:** The COVID-19 pandemic made individuals who previously had an active life through physical activities become susceptible to a sedentary lifestyle. The practice of physical exercise was reported at a rate of 30.1% before the pandemic and a decrease to 27.6% among women aged 18 to 29 years. Providing the trigger for depression and anxiety, with high rates of depression and anxiety of 23.6% and 45.1% respectively during the pandemic. **CONCLUSION:** When compared to escitalopram, fluoxetine showed greater bioavailability, as it has a greater affinity with selective serotonin transporters. However, in 2015 and 2017, escitalopram showed an increase in sales, as fluoxetine showed adverse actions and less effective results. Therefore, deeper studies involving these two drugs used for depression and anxiety are necessary.

Keywords: “Covid-19”,“depression”,“antidepressant”,“antidepressant”

ASSESSMENT OF PHENOTYPIC CHANGES IN HUMAN IPSC-DERIVED ASTROCYTES UPON IFN-Y EXPOSURE

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Introduction: Astrocytes play a pivotal role in maintaining the homeostasis of the central nervous system (CNS). Various injuries such as infections, acute traumas, and chronic neurodegenerative diseases trigger a process known as reactive astrogliosis, leading to diverse phenotypic changes encompassing morphology, gene expression, and cytokine release. **Objectives:** This study endeavors to examine the distinctive features of reactive astrogliosis following IFN-γ stimulation in human astrocytes. **Material and Methods:** Human astrocytes were derived from iPSCs by initially differentiating them into neural stem cells using a commercial induction medium. Subsequently, these cells were cultivated with N2 and fetal bovine serum for three weeks to induce a glial phenotype. Following serum deprivation for 24 hours, mature astrocytes were subjected to IFN-γ treatment. Gene expression analysis of pro and anti-inflammatory cytokines along with BDNF was carried out through PCR assays, comparing treated and untreated cells across varying concentrations of IFN-γ. **Results:** iPSCs were successfully differentiated into astrocytes. A concentration-dependent alteration in the gene expression of BDNF, IL-6, and IL-1B was observed following a challenge with IFN- γ. **Conclusion:** These findings suggest a reactive glial response to IFN-γ. However, further experiments are warranted to elucidate the specific phenotypic changes induced by this treatment in astrocytes.

Acknowledgments: CNPq, IDOR, FIOCRUZ

Keywords: Glia, astrocyte, reactive astrogliosis, inflammation, central nervous system

ACUTE GALACTOSEMIA INCREASES ACETYLCHOLINESTERASE ACTIVITY IN RAT BRAIN IN AN AGE-DEPENDENT MANNER

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Introduction: Galactosemia is characterized by a deficiency in the galactose metabolism. The main clinical findings observed in patients affected by galactosemia include developmental delay, as well as learning and memory disabilities. So far, the pathophysiology underlying the brain complications is still unclear. **Objectives:** Considering that a disruption in cholinergic neurotransmission has been related to the alterations of cognitive processes, we aimed to evaluate the effects of high galactose levels on the activity of acetylcholinesterase (AChE) in the brain of rats at different ages. **Material and Methods:** Male Wistar rats were subcutaneously injected with galactose (5 µmol/g of body weight). The control group received the vehicle (NaCl 0.9%) under the same conditions. After 1 hour, the animals were euthanized and the brains were processed according to the experimental technique. **Results:** Acute galactose administration did not alter AChE activity in cerebral cortex, hippocampus, and striatum of suckling rats (15-day-old) or adult rats (90-day-old). On the other hand, AChE activity was higher in the cerebral cortex of 30-day and 60-day-old rats receiving galactose administration, as well as in the striatum of 60-day old animals. High galactose concentrations *in vitro* also caused an increase in this enzyme activity in the cerebral cortex of 30-day-old rats. This finding was fully prevented by the combination of N-acetylcysteine plus deferoxamine and partially prevented by the presence of L-nitroarginine methyl ester or reduced glutathione in the incubation medium. Our data demonstrate that acute galactose administration causes an increase in AChE activity in the cerebral cortex of rats at certain ages. **Conclusions:** It may be therefore speculated that alterations in the cholinergic system are involved in the pathophysiology of brain abnormalities observed in galactosemic patients.

Support: FAPERJ, CNPq and UFRJ.

Keywords: acetylcholinesterase, brain, galactose, galactosemia

NUTRACEUTICAL PROPERTIES OF MANDACARU *CEREUS JAMACARU* DC., SYMBOL OF BRAZILIAN NORTHEAST, PREVENTS TOXICOLOGICAL EFFECTS OF ROTENONE

Lira, Y. V. S.[1], Nunes, R. G. S.[1], Silva. J. R. L.[2], Costa, A. R.[3], Menezes, I. R. A.[4], Santos, C. A. L.[5], Duarte, A. E.[5], Barros, L. M.[5], Souza, I. A.[6], Andrade-da-Costa, B. L. S.[1]

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Introduction: Bioactive compounds of medicinal plants from the Caatinga ecosystem have been investigated as nutraceutic agents in oxidative and inflammatory conditions. The cactus *Cereus Jamacaru* D.C. has been recognized by its medicinal properties with antitumor and cytoprotective effects on kidney and heart disease. However, it is still unknown about its potential effect on neurodegenerative diseases. **Objectives:** The present study tested the hypothesis that *Cereus jamacaru* hydroalcoholic extract can be a nutraceutic alternative for neuroprotection using *Drosophila melanogaster* model of Parkinson´s disease. **Materials and Methods**: Young flies were subdivided into 4 groups according to diet over 7 days: control, rotenone 50 µM, rotenone 50 µM + extract at 0.05 mg/g of food and rotenone 50 µM + extract at 0.075 mg/g of food. Toxicological assay was initially performed with extract concentrations ranging from 0.01 to 0.1 mg/g of diet. Locomotor activity by negative geotaxis test and survival rate were quantified. Body + head homogenates were obtained for biochemical assays of nitric oxide, free Fe^{2+}, lipid peroxidation, total thiol levels and cell viability by mitochondrial reducing capacity. **Results**: The findings indicated a rotenone-induced mortality rate of 30%, which was inhibited by dietary supplementation with *Cereus jamacaru* extract at 0.05 and 0.075 mg/g of food. The climbing index and cell viability weakening (both ~50%) and increased levels of lipid peroxidation (~33%), nitric oxide (~100%), free Fe^{2+} (~30%) as well reduced thiol (~50%) levels caused by rotenone were also reversed by the *Cereus* extract. **Conclusions**: The results corroborated our initial hypothesis that *Cereus jamacaru* in the diet exerts antioxidant effects against rotenone-induced damage. The data stimulate additional pharmacological studies to investigate *Cereus jamacaru* as a novel dietary intervention for the prevention or treatment of PD. Furthermore, the results favor the use of this natural product from the Caatinga ecosystem in a sustainable perspective.

Support: CAPES, CNPq, FACEPE

Keywords: cactaceae; mandacaru; antioxidant activity; *Drosophila melanogaster;* rotenone; nitric oxide levels

ULTRA ENDURANCE RUNNING AFTER HIGH-VOLUME TRAINING DO NOT INTENSIFY CEREBRAL CORTEX ANTIOXIDANT RESPONSE OR ASTROGLIAL REACTIVITY

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Introduction: Ultra endurance running (UER) has been associated with transient impairments in cognitive performance. Previously, we demonstrated that high-volume (HV) running training in rodents, under moderate intensity, induced cerebellar oxidative stress. When this training was followed by an exhaustion test (ET) simulating UER, GFAP isoform profile modifications suggested impaired astrocyte reactivity. **Objectives**: The current study investigated whether this vulnerability occurs in the cerebral cortex (CCx), considering phenotypic differences in the astroglia. **Materials and methods**: 60 adult male rats were divided into six groups according to the training volume and ET: control (C), control+ET (C-ET), moderate-volume (MV) training and MV-ET, high-volume training (HV) and HV-ET. The training period was 30 (MV) and up to 90 (HV) min/day, 5 times/week for 3 months (maximum velocity of 24m/min). CCx and liver homogenates were obtained. Analysis of GFAP isoform profile, reactive oxygen species (ROS) and Nitric oxide (NO) levels, NADPH-oxidase, superoxide dismutase and catalase activities were done. **Results**: MV and HV training induced higher SOD activity and NO levels compared to control condition but an additive effect of ET was only seen in the MV-ET group (SOD $p < 0,01$; NO $p < 0,05$). CAT activity was not modified by the MV or HV training neither by the ET. ROS did not differ among the groups. In the HV group, NADPH oxidase activity increased almost 202% in the liver ($p < 0,01$) but did not change in the CCx ($p > 0,05$). Levels of 39-, 42- and 45-KDa GFAP isoforms were increased by HV volume, but these levels were reversed in the HV-TE group. 50-kDa levels were raised by training alone (EV $p = 0,0038$). **Conclusions:** CCx display resilience to oxidative damage induced by high-volume training. The lack of additional antioxidant or astroglial reactivity after ET, indicates adaptive responses of astrocytes to UER.

Support: FACEPE, CAPES, CNPq

Keywords: Ultra endurance running, oxidative stress, cerebral cortex, high-volume training, catalase, superoxide dismutase, glial fibrillary acidic protein

THE PROTECTIVE EFFECT OF RUTIN ON *DROSOPHILA MELANOGASTER* ABOUT BEHAVIORAL AND BIOCHEMICAL ASPECT INDUCED BY MERCURY CHLORIDE (HGCL2)

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Introduction: Mercury chloride (HgCl2) acts as a bioaccumulator capable of causing numerous neurological and physiological changes in organisms in a negative way. However, rutin has been considered a very effective antioxidant compound in the treatment of neurodegenerative diseases, as it can neutralize radicals capable of damaging neuronal cells. **Objective:** This study aimed to evaluate rutin as a neuroprotective agent against the damage induced by HgCl2 in *Drosophila melanogaster.* **Methods:** The exposure of the flies to the agents was carried out in triplicate, and about 150 adult flies were evaluated. To assess the antioxidant action of rutin, MTT, phenanthroline, nitric oxide, total thiols and NPSH tests were carried out in the following concentrations: Control (1500 µL of distilled water), 1 mg/g of HgCl2, 0.5 mg/g of Rutin + HgCl2, 1 mg/g of Rutin + HgCl2, 2 mg/g of Rutin + HgCl2. **Results and discussion:** About the test of negative geotaxis, the result showed that flies exposed to HgCl2 had difficulties in flight. The group treated with HgCl2 alone had a high mortality rate, while in combination with different concentrations of rutin, it heard a moderate reduction in the number of deaths, as well as in the negative geotaxis data in which the rutin had a positive effect. An increase in iron (II) levels was observed at the highest concentrations of rutin, while at low concentrations, rutin significantly decreased nitric oxide levels. The HgCl2 + R group (2 mg/g) showed a significant increase in the total thiols content, while for the NPSH all rutin concentrations showed a significant increase in the levels of non-protein thiols. **Conclusion:** Our results demonstrate that mercury chloride can cause oxidative stress in D. melanogaster. However, the results suggest that rutin has antioxidant and protective effects against the damage caused by HgCl2.

Support: CAPES and CNPq

Keywords: Fruit fly, Antioxidants, Oxidative stress, ROSs

IN SILICO MOLECULAR DOCKING ANALYSIS OF ANACARDIC ACID AGAINST PRO-INFLAMMATORY TARGETS IN PARKINSON DISEASE

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Introduction: Parkinson's Disease (PD) is the second most prevalent neurodegenerative disease worldwide, but it has not been fully understood and still has no cure. Since neuroinflammatory mechanisms are associated with the onset and progression of PD, natural compounds with recognized anti-inflammatory action, such as the polyphenol Anacardic Acid (AA), have been investigated for their potential to prevent or mitigate the main symptoms. **Objectives:** Using Molecular Docking we investigated the ability of the saturated AA to interact with two risk factors responsible for aggravating the PD: the cytokine TNF-alpha and the protein 3 (NLRP3) inflammasome involved in microglial activation and maturation of IL1b and IL18 cytokines. **Material and Methods:** Briefly, the predicted 3D structures for AA and its targets were obtained from the PubChem and Protein Data Bank servers, respectively. Then, they were processed in the Discovery Studio visualizer (BIOVIDA). The DockThor server (LNCC.BR) was used to generate the complex molecular interactions between AA and its targets. Finally, the output data was then analyzed using Avogadro 2 software (Informer Technologies, Inc). **Results:** Docking values (Kcal/mol) revealed a binding affinity of -8.913 with the pro-inflammatory TNF-alpha and -6.924 with the NLRP3. The overall interaction energy was 115,684 and 28,195 for TNF-alpha and NLRP3, respectively. **Conclusion**: Our *in silico* analyses confirmed AA's ability to bind to the two PD's risk factors tested. Since TNF-alpha and NLRP3 are deregulated and associated with macrophage and microglial inflammasome activation in PD, both in humans and animal models, the residues of interactions revealed here for the AA may help to clarify the molecular mechanisms associated with its reported neuroprotection. The data also reinforce the potential of AA as a novel, natural and low-cost therapeutic approach to protect the interaction between neurons and microglia, and arrest neuroinflammation associated with a PD.

Keywords: Molecular Docking, TNF-alpha, NLRP3, Neuroinflammation, Parkinson's Disease, Anacardic Acid

Support: FACEPE, CNPq, CAPES

PREBIOTICS IMPROVE DEPRESSIVE-LIKE BEHAVIOR THROUGH THE MICROBIOTA-GUT-BRAIN AXIS MODULATION IN A MURINE MODEL OF PARKINSON'S DISEASE

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Introduction: Parkinson's disease (PD) is the second most prevalent neurodegenerative disease in the world. About 40% of patients have depression associated to motor symptoms. Braak's hypothesis postulates that PD pathology begins in the intestine and could be influenced by gut dysbiosis. From the gut, the PD pathology could reach the central nervous system through the gut-brain communication. Prebiotics are nutraceuticals that regulate the gut dysbiosis and confer benefits in the brain. **Objectives**: To evaluate the effects of prebiotics fructooligosaccharides (FOS) and galactooligosaccharides (GOS) in mice induced to PD. **Materials and methods**: C57BL/6 mice (8 weeks old) were injected with 2.5mg/Kg/day of rotenone for 20 days, parallel, also received a mixture of FOS (3mg/Kg/day) and GOS (4mg/Kg/day) orally. On the 20th day, the animals were submitted to rotarod and open field tests, sucrose preference test and tail suspension test for evaluation of depressive behavior. The animals were sacrificed, the brain was removed for analysis of western blotting, the colon was collected to immunofluorescence, and feces were collected for 16S sequencing of the gut microbiota. **Results**: Prebiotic treatment prevented motor deficits and depressive-like behavior. Prebiotics also increased tyrosine hydroxylase protein levels in the substantia nigra, reduced iba-1 levels, thereby decreasing dopaminergic neuronal loss and microglial activation, respectively. In addition, prebiotics decreasing the expression of α-synuclein in the substantia nigra. In the prefrontal cortex, prebiotics attenuated neuroinflammation by decreasing NFkB and IL1-β, and promoted neuroplasticity by increasing serotonin transporter (SERT). In the intestine, prebiotics caused an up-regulation of occludin and promoted an increase in the Bacterioidaceae and Desulfovibrionaceae families, the genus Bacterioides and the species Lactobacillus reuteri, Alistipes sp., Desulfovibrio sp. **Conclusion**: The prebiotics promotes the growth of beneficial bacteria, increase integrity of the intestinal epithelium, improved the motor and depressive symptoms of PD and reduces PD pathology in the brain.

Suport: FACEPE, PROEP, CNPQ

keywords: Parkinson's Disease, Microbiota. Prebiotics. Depression.

ANTITUMOURAL ACTIVITY OF COMPONENTS ISOLATED FROM *PERSEA FULVA* (LAURACEAE)

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Introduction: Gliomas are the most common intracranial tumors, accounting for more than 50% of all primary brain tumors, with glioblastoma multiforme (GBM) being the most common and aggressive. Due to the high lethality and the few therapeutic alternatives, there is an increasing need to find new therapeutic agents for the treatment of GBM. **Objective:** To evaluate the antiproliferative activity of alkyl γ-lactones isolated from *P. fulva* (Lauraceae) in rat C6 glioma cell lines. **Material and Methods:** The compounds majorenolide, majoranolide and majorinolide were previously isolated from the ethyl acetate extract of *P. fulva*. C6 cell lines were plated at a volume of 100 µL/well (1x106 cels/mL) in 96-well plates, treated with the compounds at different concentrations (five for each compound) for cell viability tests with MTT (-3-(4,5-dimethyl-2-thiazole) 2,5-diphenyl-2-H-bromide tetrazom), this assay evaluates the function of mitochondrial dehydrogenases in viable cells. The sample size (n) for each group was eight (8) and the experiments were carried out in triplicate. The evaluation parameter was the percentage of cell death. The concentration effective in decreasing cell viability by 50% (CE50) was calculated for all the γ-lactones by means of a concentration-response curve using Graph Pad Prisma 5.0 software. **Results:** The compounds isolated from *P. fulva* were able to decrease the cell viability of rat glioma cell lines (C6), through a concentration-dependent mechanism after 24 hours of exposure to the compounds. The compound majoranolide (CE50= 6.69 µM) was the most potent, followed by majorenolide (CE50= 12.04 µM) and majorinolide (CE50= 41.90 µM). **Conclusions:** this work contributes to the chemical and biological study of the species *P. fulva*, which belongs to the Lauraceae family. The γ-lactones isolated from *P. fulva* showed promising antiproliferative activity in rat glioma cells, but studies to determine the mechanism of cell death and *in vivo* investigations to prove anticancer activity are needed.

Keywords: *Persea fulva*; MTT; glioma.

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EFFECT OF NICOTINE IN DAMAGED ASTROCYTES: PROTECTIVE EFFECTS FOR PARKINSON'S DISEASE

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Introduction: The role of astrocytes in Parkinson's disease (PD) pathogenesis is not fully clear. Some pre-clinical studies have shown their involvement in neuroinflammation, on the other hand, aminochrome, an ortho-quinone formed during dopamine oxidation to neuromelanin, is able to induce astrocyte death, and alpha-synuclein immunoreactive astrocytes are present in post-mortem PD patients' samples. PD is still a disease without a cure and the search for neuroprotective compounds is current. Nicotine, the main compound from *Nicotiana tabacum*, is able to protect neurons against aminochrome toxicity; however, the effect in dysfunctional astrocytes was not investigated. **Objectives**: In this study, we evaluated the effect of nicotine on glial cells expressing human alpha-synuclein and/ or treated with aminochrome. **Methodology:** We used primary culture of mesencephalic cells from rat embryos (E15) and Wild-type U251 human astrocytes or U251 transfected for A53T alpha-synuclein – nYFP super-expression. These cells were treated with nicotine and/ or aminochrome. The cell viability was assessed by MTT test or propidium iodide test, cell morphology was accessed by Rosenfeld's staining, and analysis of autophagy dysfunction was performed by Western Blotting for LC3 and P62 astrocytes from cell lineage or submitted to treatment with nicotine and/ or aminochrome. The MTT test was performed to assess the cell viability. **Results:** We observed that nicotine induces vacuolation and protects cells in primary cultures against aminochrome neurotoxicity. We also observed that 10 µM nicotine for 6 h increased the expression of LC3II in wild-type U251 cells. Moreover, nicotine (0.1 – 50 µM, for 48 and 72h) protects transfected alpha-synuclein U251 cells against aminochrome cytotoxicity. **Conclusion:** Nicotine is protective for damaged astrocytes. These results suggest the activation of autophagy by nicotine. More analysis is required to investigate the role of autophagy as a mechanism of neuroprotection.

Keywords: Parkinson's disease; astrocytes; nicotine; neuroprotection.

Support: CNPq; Fapesb

INVESTIGATION OF THE NEUROPROTECTIVE AND ANTI-NEUROINFLAMMATORY EFFECTS OF TERPENE COMPOUNDS IN AMYOTROPHIC LATERAL SCLEROSIS

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INTRODUCTION: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects nerve cells in the brain and spinal cord, causing gradual loss of muscle function. Glial cells, such as astrocytes and microglia, play an important role in promoting and reversing the inflammatory process in the central nervous system. Therapeutic strategies aimed at reducing inflammation in the nervous system have been investigated in the development of neuroprotective drugs, as well as terpenes, natural substances derived from plants. Lupeol and Ursolic Acid are examples of terpenes that have anti-inflammatory and neuroprotective activities, being able to modulate the inflammatory response in the nervous system, stimulating the production of pro-inflammatory mediators and promoting the expression of neurotrophic and anti-inflammatory factors. **OBJECTIVES:** Investigate the neuroprotective and anti-neuroinflammatory effects of the terpenes Lupeol, Caurenoic Acid, Ursolic Acid, Oleanolic Acid and Methyl Careonate. **METHODS:** Cultures of PC12 neuronal cells were treated with Lupeol, Ursolic, Xylopic, Caurenoic, Oleanolic and Methyl Caureonate acids in concentrations from 0.1 to 100 µM, and cell viability was determined after 72 h of treatment by the MTT test. **RESULTS:** PC12 cells treated with Lupeol had an increase in cell viability at both concentrations adopted (0.1 to 100 µM). Caurenoic Acid, Ursolic Acid, Oleanolic Acid and Methyl Careonate showed increased viability at concentrations from 0.1 to 50 µM according to the MTT. However, Xylopic Acid showed an increase in cell viability only at concentrations of 0.1, 1 and 10 µM when compared to the control. **CONCLUSION:** These results encourage the continuation of in vitro studies with these compounds and their potential application in the therapy of Amyotrophic Lateral Sclerosis.

KEYWORDS: Neuroinflammation, Neuroprotection, Anti-inflammatory, Terpenes

SUPPORT: CAPES, PGNeT, INNT

EVALUATION OF GASTROINTESTINAL MOTILITY AND WEIGHT IN AN AMININOCHROME ANIMAL MODEL OF PARKINSON'S DISEASE

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Introduction: Parkinson's disease (PD) is a slowly progressive neurodegenerative disease, with clinical presentation of bradykinesia, muscle rigidity, and resting tremor. Patients also experience constipation in the early stage of PD, which may be caused by changes in the enteric nervous system. *In vitro* and *in vivo* study models revealed aminochrome, a compound derived from dopamine oxidation, as an inductor of cellular and molecular changes present in patients with the disease. However, alterations in enteric nervous system were never investigated. **Objectives:** The objective of this study was to evaluate changes in intestinal transit time and weight of male Wistar rats with aminochrome-induced PD, after previous characterization of the damage by analyzing the fluorescence intensity of tyrosine hydroxylase in the substantia nigra pars Compacta (SNpc) and ventral tegmental area (VTA). Furthermore, to evaluate the effect of the flavonoid rutin on the neuroprotection of the SNpc and on intestinal transit. That away, the animals were randomized into four groups: control group, aminochrome, rutin, and aminochrome + rutin. The rutin was administered by gavage for 21 days. The live weight of the animals was evaluated from the beginning to the end of treatment, and the intestinal transit time was evaluated using the carmine red test. **Results:** the aminochrome did not generate statistical changes in intestinal transit, but caused loss of dopaminergic neurons in the SNpc, but not in the VTA, and rutin prevented this loss, in addition to accelerating intestinal transit time and promoting modulation in the animals' weight. **Conclusions:** Aminochrome caused a soft slowing of intestinal transit, and death of dopaminergic neurons, which was prevented by rutin.

Support: FAPESB.

Keywords: Parkinson's disease, neurodegeneration, rutin, neuroprotection, gut.

THE EFFECTS OF FLAVONOID RUTIN ON OXYGEN AND GLUCOSE DEPRIVATION MODEL IN PRIMARY CULTURES OF RODENT RETINA.

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Introduction: According to the World Health Organization there's been an increase in blindness and low vision across the world. Retinal diseases can result in these outcomes through the loss of neurons in the retina. Retinal detachment (RD) is a common condition that results in the death of neurons, even if it's treated surgically, leading to loss of vision or blindness in many cases. Previous studies have demonstrated the potential of rutin as a neuroprotective, neurogenic, and immunomodulatory compound. These findings suggests that the flavonoid rutin could be a prime candidate for the prevention of neuronal cell death in the retina during RD. **Objectives:** In this study we aim to establish a new primary cell cultures of mice retina in the laboratory and investigate the neuroprotective and immunomodulatory properties of rutin in an *in vitro* model of retinal detachment. **Material and Methods:** Once the primary mice retina cell cultures are established the cultures will be treated under an *in vitro* model of RD, the oxygen-glucose deprivation (OGD) model. In this model the cells are cultured with a OGD buffer in a hypoxic incubator. The first group of cultures will be treated with rutin during the OGD model and after, for 14 days. The second group will be treated only after the OGD model. Afterwards it will be evaluated the viability of neurons treated with rutin and the immune response through immunophenotyping focusing on cytokines that are reportedly important during RD, such as IL-33 and IL-4. **Expected Results:** It's expected to see a higher viability of neurons in the cell cultures treated with the rutin during and after OGD treatment, followed by the cultures only treated after the OGD model. It's also expected that the immune response will be lessen in the treated groups.

Support: CAPES, FAPESB, CNPq

Keywords: Retina, Rutin, Retinal detachment

THE FLAVONOIDS AGATHISFLAVONE AND APIGENIN AVOID THE DEGENERATION OF OLIGODENDROCYTES' PROCESSES IN A SIMULATION OF ISCHEMIC INJURY.

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Introduction: The loss of myelin sheath leads to neuronal death causing demyelinating neurodegenerative pathologies. One of the causes of oligodendrocytes death and demyelination is ischemic injury. The protective characteristics of flavonoids, namely agathisflavone and its monomer apigenin, have already been demonstrated. Both flavonoids reveal important effects upon controlling the inflammation through modulation of neuroinflammatory response of microglia. In addition, flavonoids can be considered as proactive against oxidative damage in hypoxia events due their antioxidant properties. **Objective:** The objective of this study was to assess the protective effects of flavonoids apigenin and bis-apigenin on morphology and viability of oligodendrocytes in an *ex vivo* model of acute ischemia. **Methods:** In this study cerebellum slices of p8–p12 mice transgenic EGFP reporter to sox-10 gene was used to identify the oligodendrocyte lineage. The flavonoids were administered in a preventive manner 60 minutes before the ischemic damage, which was induced by deprivation of oxygen and glucose for 60 minutes, and the control group was kept in oxygen-glucose normoxia. **Results:** It was observed that one hour of deprivation of oxygen and glucose did not result in a significant decrease in the density of oligodendrocytes in the granulocyte layer. Hypoxia altered the morphology of oligodendrocytes, however, ischemic damage did not reduce their numbers. The damage caused a decrease in the proportion of cells with many processes and an increase in the proportion of cells with few processes, which can have an impact on their myelination capacity. Pre-treatment with agathisflavone (10 µM) prevented the loss of processes in hypoxia-induced damage. Moreover, pre-treatment with apigenin (10 µM and 15 µM) increased the proportion of oligodendrocytes with more processes in the slices subjected to the hypoxia-induced damage. Despite changes in the morphologie of oligodendrocytes caused by ischemic damage, which was demonstrated by a decrease in processes per cell, it was observed that depriving the cells of oxygen and glucose for one hour did not reduce the number of cells expressing sox-10 in the white matter region. This region is known for having a high level of myelination. **Conclusion:** According to this study, flavonoids induce a distinct response despite their structural morphologie (dimer and respective monomer). Agathisflavone was able to decrease the effect of hypoxia on branching oligodendrocytes, whereas apigenin was found to be more effective in this response after injury.

Keywords. Ischemia, Demyelination, Flavonoids

Financial support. FAPESB; CAPES

CHARACTERIZATION OF ANTITUMORAL AND IMMUNOMODULATORY MECHANISMS OF THE FLAVONOID RUTIN IN GLIOMA CELLS IN INTERACTION WITH MICROGLIA AND ITS RELATIONSHIP WITH MIRNA EXPRESSION

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Introduction: Glioblastoma (GBM) is the most aggressive and treatment-resistant brain tumor. Interactions with microglia in the GBM microenvironment lead to dysregulation of cytokines, chemokines, and miRNAs, contributing to angiogenesis, proliferation, anti-apoptosis and chemoresistance. Rutin, a flavonoid, has shown potential in inhibiting rat glioma cell growth by activating microglia and producing pro-inflammatory mediators, but the underlying mechanisms are unclear. **Objectives:** This study aims to clarify rutin's antitumor and immunomodulatory mechanisms in human GBM cells through interactions with human microglia and their impact on miRNA expression. **Methods:** We used human GBM GL15 cells and human microglia C20. Cell viability was assessed using the MTT assay in both cell types with and without rutin treatment (1-50 µM) for 24h. miRNA-125b expression in GL15 and their secretome was evaluated 24h post rutin treatment (30 µM) using RT-qPCR. mRNA expression for cytokines IL-1β, IL-6, IL-10, TNF, and the signaling protein STAT3 in C20, treated with conditioned medium from GL15 under control conditions (MCGC) or treated with conditioned medium from GL15 treated with rutin (MCGR), was assessed 24h after treatment via RT-qPCR. STAT3 protein expression in GL15 and C20 under different conditions was examined by Western blot, while cell morphology was analyzed through phase microscopy. **Results:** Rutin treatment (30-50 µM) significantly reduced GL15 viability by approximately 50% after 24h, while C20 viability remained unaffected. Furthermore, rutin treatment of GL15 significantly reduced miR-125b expression and STAT3 protein levels. Conversely, C20 exposed to MCGR exhibited morphological changes suggestive of reactivity and showed reduced mRNA expression for IL-6, TNF, and STAT3, along with decreased STAT3 protein levels. **Conclusion:** This study reaffirms rutin's potential as an antiglioma agent and highlights its role in modulating miRNA-125b expression. This modulation, through indirect interaction with microglia, likely contributes to altering the inflammatory profile in these cells, promoting a more responsive antitumor phenotype.

Support: CNPq, INCT-Translational Neuroscience, CAPES.

Keywords: glioblastoma, microglia, miR125b, rutin, inflammatory cytokines.

AGATISFLAVONE FLAVONOID REGULATES MIR146A AND MIR155 AND THE NEUROINFLAMMATORY RESPONSE IN B-AMYLOID-STIMULATED HUMAN MICROGLIA.

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Introduction: The control of microglial activation and the neuroinflammatory process are strategies that have been investigated in the development of new therapeutic approaches for neurodegenerative diseases (NDD). Agathisflavone, a biflavonoid purified from the leaves of Cenostigma pyramidale (Tul.), has demonstrated anti-inflammatory and neuroprotective properties in in vitro and ex vivo models of NDD. **Objective:** Here, we investigated in human microglial cells, the effects of agathisflavone in modulating expression of microRNAs and inflammatory mediators, after inflammatory stimulus with β-amyloid oligomers. **Methods:** For this, cultures of human microglia of the C20 lineage were exposed to oligomers of the β-amyloid peptide (500 nM) for 4 h or to lipopolysaccharide (LPS, 1 µg/mL) for 24 h and then treated or not with agathisflavone (1 µM) for 24 h. **Results:** We observed that β-amyloid and LPS-induced microglia cultures to assume an activated inflammatory state, with increased expression of miR-146a and miR-155 and inflammatory mediators IL1-β, IL-6, and NOS2. However, in cells exposed to inflammatory damage and treated with agathisflavone, we observed a significant reduction in the concentration of miR146a and miR-155, as well as the inflammatory cytokines evaluated. We also observed in cells stimulated only with β-amyloid, an increase in the p-STAT3/STAT3 signaling protein ratio, and in cells stimulated with β-amyloid and treated with flavonoid, there was a reduction in the p-STAT3/STAT3 ratio. **Conclusion:** Thus, these data reinforce the anti-inflammatory effect of agathisflavone, highlighting its potential in the regulation of miRNAs associated with neuroinflammation, and its potential as a promising molecule for the treatment or prevention of NDD.

Keywords: agathisflavone, human microglia, miRNAS, anti-inflammatory effect.

Support: CNPq, INCT-Translational Neuroscience, CAPES.

NARINGENIN'S ANTITUMOR MECHANISMS AND AHR-DRIVEN IMMUNOMODULATION IN GLIOMA THERAPY

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Introduction: Conventional treatment for GBM includes surgical resection, radiotherapy, and chemotherapy. However, characteristics of the tumor, such as rapid proliferation and induction of immunosuppression, contribute to its poor prognosis and recurrence. In addition, the transcription factor, aryl hydrocarbon receptor (AhR), constitutively activated in tumor cells, is related to chemo and immunoresistance. AhR activation promotes cell differentiation and increases the expression of resistance genes, placing AhR antagonism as a target in cancer chemotherapy. The demonstrated antitumor and immunomodulatory effects of flavonoids point to the pharmacological potential of these drugs in the treatment of glioblastoma (GBM). Flavonoids may act as AhR antagonists and reduce the viability of tumor cells. **Objectives:** The aim of this study is to define the antitumor mechanisms of flavonoid naringenin and the possible association of its anti-glioma activity with AhR antagonism. **Material and Methods:** Naringenin was tested as an AhR antagonist at increasing non-cytotoxic concentrations (5-30µM) using the induction of CYP1A1 mediated EROD activity assay in MCF7 cells, as a marker of AhR- responsiveness. Human U87 GBM cells were exposed to naringenin (30µM) in the presence or not of the AhR agonist Indole-3-carbinol. After 24h treatment cell viability was determined by MTT and SRB essays and cell migration was determined until 48h post-treatment. **Results:** Our results demonstrated that naringenin has potent inhibitory effects on AhR activity. Furthermore, our data revealed that naringenin reduced cell viability and migration in a dose- and time-dependent manner. Additionally, it was demonstrated that the combination of naringenin and AhR agonist increased cytotoxicity to GBM cells. **Conclusions:** The characterization of the molecular mechanisms of flavonoid naringenin featuring an antagonistic effect on AhR and its role in chemosensitivity will contribute to sustaining its application as an adjuvant for GBM treatments.

Acknowledgments: CAPES, CNPq, and FAPESB.

Keywords: flavonoids, glioblastoma, aryl hydrocarbon receptor

MECHANISMS INVOLVED IN THE THERAPEUTIC EFFECT OF CANNABINOID COMPOUNDS ON GLIOMAS: A REVIEW WITH EXPERIMENTAL APPROACH

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Introduction: Brain tumors have high morbidity and mortality rates, accounting for 1.4% of all cancers. Gliomas are the most common primary brain tumors in adults. Currently, several therapeutic approaches are used; however, they are associated with side effects that affect patients'quality of life. Therefore, further studies are needed to develop novel therapeutic protocols with a more favorable side effect profile. In this context, cannabinoid compounds may serve as potential alternatives. **Objective:** This study aims to review the key enzymatic targets involved in glioma pathophysiology and evaluate the potential interaction of these targets with four cannabinoid derivatives through molecular docking simulations. Materials and Methods: Molecular docking simulations were performed using four cannabinoid compounds (Δ-9-tetrahydrocannabinol – Δ-9-THC, cannabidiol - CBD, cannabigerol - CBG, and cannabinol - CBN) and six molecular targets associated with glioma pathophysiology (Epidermal Growth Factor Receptor - EGFR, Phosphatidylinositol 3-kinase - PIK3, Fibroblast Growth Factor Receptor - FGFR, BRAF, Telomerase Reverse Transcriptase - TERT, and Indoleamine 2,3-dioxygenase 1 - IDO1). **Results:** For the EGFR factor, the compound CBG presented a negative score value and very close to the positive control, which possibly indicates a high affinity for this target. Three compounds (CBG, CBN and CBD) showed greater affinity for the PI3K target when compared to the positive control LY29400. For the TERT enzyme, the compound Δ-9-THC showed the lowest value of free energy, thus demonstrating a greater affinity for it when compared to the positive control. For the other targets (IDO1, FGFR, and BRAF), the compounds did not show affinity close to the positive control, which possibly indicates the absence of interaction through the studied pathways. **Conclusions:** The evaluated compounds exhibited favorable interactions with the analyzed enzymatic targets, thus representing potential candidates for further in vitro and in vivo studies.

Support: CAPES, CNPq, PROEX-UFPB.

Keywords: Gliomas. Cannabinoid compounds. Antitumoral. Cheminformatics Review. Experimental.

LINALOOL AND ITS DERIVATIVES AGAINST PARKINSON'S AND ALZHEIMER'S DISEASE: A REVIEW WITH AN EXPERIMENTAL APPROACH

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Introduction: Alzheimer's and Parkinson's are neurodegenerative disorders that affect a great number of people around the world, seriously compromising the quality of life of individuals, due to motor and cognitive damage. Current pharmacological treatment for such diseases aims only to reduce symptoms, in an attempt to reduce patients' suffering. This emphasizes the need to discover alternative molecules for use in prevention. In this sense, linalool becomes a possible therapeutic alternative, due to its neuroprotective effect with anti-inflammatory and antioxidant activity. **Objective:** Using pharmacokinetic studies and molecular docking, this review aimed to evaluate the toxicity, oral absorption, bioavailability, anti-Alzheimer's and anti-Parkinson's activity of linalool, as well as their derivatives. **Methodology:** Before performing Molecular Docking simulations, the compounds' pharmacokinetic characteristics were evaluated. For Molecular Docking, 10 compounds derived from linalool, and molecular targets involved in Alzheimer's and Parkinson's pathophysiology were selected. **Results:** According to the Lipinski rules, the compounds under study exhibited good oral absorption and bioavailability. Some tissue irritability was observed regarding toxicity. Concerning Parkinson-related targets, the linalool-derived compounds demonstrated excellent binding affinity for α-Synuclein, Adenosine Receptors, Monoamine Oxidase (MAO), and Dopamine D1 receptor proteins. Out of the 10 compounds tested, the best performance was observed in Linalyl (L2), 8-oxolinalyl acetate (L4), and 8-carboxylinalyl acetate (L5), which showed a higher probability of binding to the D1 receptor, MAO enzyme, COMT α-Synuclein enzyme, respectively, compared to the positive control. Regarding Alzheimer's disease targets, only 8-hydroxylinalyl acetate (L3) showed promise against BACE enzyme activity. **Conclusion:** The compounds studied presented excellent pharmacokinetic parameters, oral bioavailability, low genomic toxicity and presented a high modulatory probability of the diseases studied, being potential candidates for future medicines.

Support: CAPES, CNPq, PROEX-UFPB.

Keywords: Monoterpenes. Neurodegeneration. Molecular Docking.

ANTIDEPRESSANT POTENTIAL OF AN EUGENOL DERIVATIVE IN A MURINE BEHAVIORAL MODEL

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Introduction: Depression is a serious and potentially life-threatening mood disorder. Its etiology still remains unclear, however, it is believed that susceptibility to depression is influenced by a variety of genetic, epigenetic, endocrine and environmental risk factors. Essential oils are reported to regulate brain health and functions associated with mood and neurodegeneration. Eugenol derivatives have been associated with reversal of depressive-like behavior, as well as an antinociceptive and anti-inflammatory effect. **Objective:** The goals of this paper were to examine the antidepressant activity of an eugenol derivative in an animal model of depression in behavioral tests. **Methodology:** This project used female Swiss albino mice (n=8), previously approved by the Ethics Committee on the Use of Animals (CEUA) under protocol no. 5029280422. The methodology used consists of inducing experimental depression through repeated administration of dexamethasone in mice, as well as treating the same mice with the eugenol derivative at doses of 25, 50 and 100 mg/kg (v.o.), followed by behavioral evaluation of its antidepressant effect through the sucrose preference test (SPT) and the tail suspension test (TST) on the 15th day. **Results:** The results obtained demonstrate the antidepressant potential of the eugenol derivative through the significant increase in the latency to immobility at a dose of 100 mg/kg ($p<0.0001$), and the reduction in the total immobility time in the tail suspension test ($p <0.01$), as well as absence of anhedonia in the sucrose preference test. **Conclusion:** In summary, it is possible to conclude the potential antidepressant effect of the eugenol derivative at a dose of 100 mg/kg, as well as to display the efficacy of the dexamethasone protocol for the induction of experimental depression. In addition to contributing to a better pharmacological understanding of the drug, providing guidance for subsequent research.

Support: CAPES, CNPq, PROEX-UFPB

Key words: Eugenol, depression, behavior, essential oils.

TETRAHYDROLINALOOL MODULATES LPS-INDUCED MICROGLIA/ MACROPHAGE ACTIVATED PHENOTYPE

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Introduction: Tetrahydrolinalool (THL) is a cyclic monoterpenoid alcohol, originated during the metabolism of linalool, a constituent of various essential oils. Currently, THL has been reported because its anxiolytic and antidepressant potential, which seems to involve glutamatergic, GABAergic, oxidonitrergic and anti-inflammatory pathways. **Objective:** Based on these findings, pioneering studies have been developed in order to understand the protective effect of THL in microglial cultures against inflammatory damage, an important finding in psychiatric disorders such as anxiety and depression. **Methodology:** At present we investigated the effects of THL (8, 16, 32, 64 µM) on the viability and activation of microglia submitted or not to inflammatory stimulus induced by LPS (1 µg/mL). Cytotoxicity was determined by MTT assay, and: the phenotype-related activation was analyzed by interference phase contrast microscopy 24 h after the treatments. **Results:** treatment alone had no effect on microglia viability, nor morphology, which presented a multipolar and branched quiecent-like phenotype, with thin and long processes extending from small and rounded cell bodies. In contrast, LPS-treated cultures presented an increase in MTT metabolism and microglia cells displayed a rounded or amoeboid morphology, with a reduction and retraction of their cytoplasmic processes, which were less numerous, shorter, and thicker; these morphological changes were reduced by simultaneous treatment with LPS and THL and ramified microglia were more evident. Quantification of microglial morphology confirmed such alterations and demonstrated a significant increase in the relative density of amoeboid microglia following LPS treatment, which was significantly reduced in the presence of THL. **Conclusion:** These results demonstrate, at least in part, that THL seems to modulate microglial activation and proliferation. However, more studies are under investigation to better characterize THL immunomodulatory effects in microglia and the possible impact in neuroprotection in neuroinflammatory conditions.

Support: CAPES, CNPq, PROEX-UFPB.

Keywords: Neuroinflammation. Depression. Anxiety. THL

CHRONIC GALACTOSE ADMINISTRATION IMPAIRS CEREBELLUM DEVELOPMENT OF RATS

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Introduction: Galactose is a monosaccharide used as an energy source and as a precursor for glycosylation. A deficiency of one enzyme of the Leloir pathway causes galactosemia. Symptoms begin after ingestion of breastmilk and patients present signs of intoxication that may progress to death. Current treatment is based on a diet with exclusion of galactose, which does not prevent long-term complications. The main clinical findings include developmental delay, speech problems and movement disorders. **Objectives:** The aim of this work was to investigate the impact of galactose in rat cerebellar development. **Material and methods:** Male and female Wistar rats were subcutaneously injected from the 5th to the 17th day of life with galactose (5 µmol/g of body weight, every 12 hours). Under the same conditions, animals from the control group received the vehicle (NaCl 0.9%). Twenty-four hours after the last injection, the animals were euthanized and the samples were processed according to the experimental techniques. **Results and Discussion:** A decrease in the size of the cerebellum and disorganization of its layers were observed in the animals treated with galactose. GFAP protein was evaluated in order to analyze the morphology of astrocytes and Bergmann glia. Immunofluorescence images for GFAP suggest alterations in Bergmann glial fibers. GFAP, TrkB and p-CREB (Ser133) immunocontent were unaltered in cerebellar *vermis* of animals receiving galactose. **Conclusion:** The morphological changes found herein indicate a failure in cerebellar cell migration. It may suggest that galactose plays a role in the cerebellar changes observed in galactosemic patients. However, the molecular mechanisms involved in these alterations are yet to be clarified.

Support: CNPq, FAPERJ, UFRJ.

Keywords: cerebellum, galactose, galactosemia.

MINOCYCLINE AS A NEUROPROTECTIVE AGENT IN AN ANIMAL MODEL OF MAPLE SYRUP URINE DISEASE

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Introduction: Maple Syrup Urine Disease (MSUD) is a rare genetic disorder characterized as a deficiency of the branched-chain α-keto acid dehydrogenase enzyme complex, resulting in tissue and body fluids accumulation of branched-chain amino acids (BCAA) and their corresponding alpha-keto acids. Evidence from the literature support an important role for amino acid imbalance in the brain, leading to impaired synthesis of brain proteins and neurotransmitters, resulting in neurological disorders, learning and memory. **Objectives:** The present study aimed to evaluate the effects of minocycline in behavioral and neurochemical parameters on rats submitted to an animal model of MSUD. **Methods:** Wistar rats were divided in 4 groups; 1) control, 2) MSUD (BCAA), 3) minocycline, and 4) MSUD + minocycline (BCAA + minocycline). A pool of BCAA (15,8 µL/g) was administrated subcutaneously, twice a day, from the 7th to 28th postnatal days in groups 2 and 4. Minocycline (50 mg/kg) was administrated via gavage, once a day in groups 3 and 4. Open field habituation memory test, choline acetyltransferase (ChAT), acetylcholinesterase (AchE) and oxidative stress parameters were evaluated. **Results and Conclusions:** The administration of BCAA inhibited ChAT activity and increased AchE activity; minocycline reversed these effects. Markers of lipid and proteins oxidative damage and production of free radicals were increased by BCAA administration; all these effects were also reversed by minocycline. The present results suggest that minocycline may present a protective effect in MSUD.

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Keywords: Maple Syrup Urine Disease, oxidative stress, choline acetyltransferase, acetylcholinesterase.

CITRONELLAL AND ITS DERIVATIVES AGAINST PARKINSON'S DISEASE AND ALZHEIMER'S DISEASE: A REVIEW WITH AN EXPERIMENTAL APPROACH

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Introduction: Parkinson's and Alzheimer's disease, prevalent neurodegenerative diseases, affect movement and memory, with no known cures, posing significant challenges in research and healthcare.Citronellal, a monoterpene generated through the methylerythritol phosphate (MEP) biosynthetic pathway of plant secondary metabolism. Therefore, studies have shown that citronellal, in the central nervous system, has neuroprotective activities, as it is a GABAA agonist, can modulate glutaminergic receptors and has anti-inflammatory action, as it can inhibit neuroinflammatory response pathways, which are important to prevent the emergence of AD and PD conditions. **Objective:** The study is developed with the aim of analyzing the enzymatic targets related to the pathophysiology of Alzheimer's disease (AD) and Parkinson's disease (PD) and evaluating the possible interaction of these compounds based on an experimental review. **Methodology:** The software MarvinSteck, HyperChem, OSIRIS Data Warrior, and Special Data File were used, which enabled the analysis of the therapeutic potential of the monoterpene. The molecules of the 7 chemical compounds derived from citronellal were obtained through a literature review. **Results:** Regarding oral absorption, four compounds reached the maximum absorption rate. In the toxicity analysis of the substances, risks of irritability were found in five citronellal derivatives, however, no genetic toxicity was observed, the pharmacokinetic parameters obtained obeyed Lipiniski's rules. For the targets analyzed in this study of Parkinson's disease, A-Sinkein, adenosine receptor A24 and the molecular fitting with a satisfactory interaction with citronelic acid compounds (C4), metoxicythronel (C1), C4, C1, respectively. In Alzheimer's, citronellal derivatives showed an affinity only for the BACE enzyme. **Conclusion:** The compounds studied presented considerable parameters in terms of their pharmacokinetics, and a high probability of binding with targets associated with the diseases studied. Therefore, more studies are needed on this substance as a therapeutic target for Alzheimer's and Parkinson's diseases.

Key words: Alzheimer's disease, parkinson, citronellal, molecular docking.

Support: CAPES, CNPq, PROEX-UFPB.

INVOLVEMENT OF THE GLUTAMATERGIC AND OXIDONITRERGIC SYSTEMS IN THE ANTIDEPRESSANT AND ANXIOLYTIC EFFECT OF PHENYLPROPANOID ADMP

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Introduction: Depressive and anxiety disorders are among the most common mental health conditions worldwide. Stress is the main environmental factor that can cause hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA), an important mechanism in the response to stress. ADMP is a phenylpropanoid that has shown high antioxidant activity in previous studies, a relevant property for antidepressant drugs. **Objective:** Was to investigate the antidepressant and anxiolytic activity of ADMP in mice submitted to the model of stress induced by dexamethasone and possible routes of action. **Methods:** In order to investigate the pharmacological activity, studies *in silico* and *in vivo* were carried out. The animals were pre-administered with dexamethasone (64µg/kg sc) 4h before conducting the behavioral tests, with ADMP (25, 50 and 100 mg/kg ip) and imipramine (10 mg/kg ip) administered 45 and 30 minutes respectively before of the tests. **Results:** The ADMP showed lower binding energy for the L-arginine/NO/cGMP pathway and the NMDAR receptor, showed no toxicity in the parameters of mutagenicity, carcinogenicity, toxicity of the reproductive system, irritability of the skin tissue in the *in silico* study. In *in vivo* studies, the administration of ADMP produced an antidepressant effect and anxiolytic without altering locomotor and exploratory activity at the dose of 50 mg/kg.The results suggest that the antidepressant activity may be dependent on the inhibition of mediators of the NMDA-L-arginine/NO pathway. CGMP. In the evaluation of oxidative parameters, an increase in GSH levels was observed in animals submitted to treatment with ADMP at doses of 25 and 100 mg / kg compared to the group treated with dexamethasone. **Conclusion:** Then treatment with ADMP produced antidepressant and anxiolytic effects, through the involvement of the NMDA receptor and mediators of the L arginine/NO/cGMP pathway.

Support: CAPES

Keywords: Non-clinical study, ADMP, phenylpropanoid, mechanism of action.

THE ROLE OF GLIAL CELLS IN NEUROINFLAMMATION IN THE CONTEXT OF ALZHEIMER'S DISEASE

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Introduction: Alzheimer›s disease (AD) is the world's leading form of dementia. The pathogenesis of AD includes increased brain levels of β-amyloid peptide oligomers (AβOs). The increase in oligomer concentration is neurotoxic and causes, among the damages, synapse loss, mitochondrial dysfunction and impairment of proteostasis mechanisms. Neuroinflammation, notably the activation of microglia and astrocytes to pro-inflammatory states, has been associated with the pathogenesis of several neurodegenerative diseases. The current work aimed to evaluate the neuroprotective role of glial cells in the context of the pathophysiology of AD. **Method:** This study aims to investigate potential modulations in morphology and function of glial cells in experimental models of AD, evaluating the *in vitro* activation of microglia promoted by AβOs , and *in vivo* microglia and/or astrocytic activation in mice receiving intracerebroventricular (icv) administration of AβOs. Expression of cytokines and glial markers were analyzed by RT-PCR and Immunocytochemistry. Preliminary results suggest an increase in glial activation markers, such as GFAP, GLAST, IBA1, F4/80, and complement immune system markers such as C1q, 7 and 4 days after i.c.v. infusion of AβOs. No significant differences were observed in the expression of TNF-α. Immunocytochemical analyzes suggest greater nuclear factor-κB translocation in microglial cells treated with AβOs. Results are consistent with an important role of glial cells in the pathogenesis of AD, with glial activity altered and activated in both models. In the face of chronic processes, these cells can remain in an activated state, which may contribute to exacerbate Aβ pathology.

Support: CAPES, CNPq, FAPERJ, INNT

Keywords: Alzheimer's disease, glial cells, neuroinflammation

CHRONIC HYPERPHENYLALANINEMIA CAUSES NEUROCHEMICAL AND BEHAVIORAL ALTERATIONS IN FEMALE RATS.

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Introduction: Phenylketonuria (PKU) is an autosomal recessive inborn error of amino acid L-phenylalanine (Phe) metabolism caused by the deficiency of phenylalanine hydroxylase (PAH) activity. Biochemically, it is characterized by hyperphenylalaninemia (HPA). Clinically, patients present psychomotor impairment and severe intellectual disability. The pathogenesis of brain alterations related to PKU is based on the neurotoxicity exerted by HPA, which is not completely understood. In this context, the aim of this study was to evaluate neurochemical and behavioral parameters in rats submitted to an experimental HPA model. Five-day-old female Wistar rats received 2 daily subcutaneous administrations of Phe (5.2 µmol/g; 12 h interval between administrations) and one daily subcutaneous administration of p-chlorophenylalanine (0.9 µmol/g), a PAH inhibitor, from 5th to 30th day of life. Control group received saline solution under the same conditions. Twenty-four hours after the last administration, behavioral tasks (open field and radial maze) were evaluated. Immediately after, animals were euthanized and cerebral cortex, hippocampus and striatum were harvested and used for the determination of the levels of brain-derived neurotrophic factor (BDNF) and cytokines and synaptophysin immunocontent. It was observed that HPA caused cognitive deficit in animals in the open field test, without any alteration of the radial maze. It was also found a decrease in immunocontent of synaptophysin, a marker of synaptic integrity, and an increase in interleukin 6 levels in cerebral cortex of hyperphenylalaninemic animals. On the other hand, BDNF levels were not altered. The present study demonstrated that chronic HPA caused cognitive damage, which could be explained by synaptic changes and neuroinflammation. These results may contribute to the understanding of the pathological mechanisms of cognitive damage observed in phenylketonuric patients.

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FLAVONOID RUTIN REDUCES TOXICITY OF ABERRANT CELLS WITH SOD1G93A MUTATION AND PROMOTES MOTONEURON SURVIVAL

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INTRODUCTION: Amyotrophic Lateral Sclerosis (ALS) is characterized by motor neuron degeneration and consequent loss of motor function. ALS's pathological mechanism is still unclear, but mutation in specific genes like SOD1 can provide good drug screening models. Rats expressing human SOD1G93A mutation present transformed microglia with astrocyte phenotype (Aba cells) that are highly toxic to motoneurons. Rutin is a natural flavonoid with remarkable biological activities in the central nervous system cells, such as antitumoral, and neuroprotective via anti-inflammatory, antioxidative, and modulatory effects in glial cells. **OBJECTIVE** The aim of this study was to characterize the action of the flavonoid rutin in Aba cells and its consequence on motoneuron viability. **METHODS** Aba cells were collected from symptomatic rats, cultured in DMEM, and treated with DMSO as vehicular control or Rutin (20 micromolar) for 24h. To evaluate Aba cells proliferation Immunocytochemistry was performed to S100B and Ki67. Data were analyzed and quantified with confocal microscopy and ImageJ. After that, motoneuron was cultivated from rat embryos (p15) and treated with conditioned media (CM) from Abas treated with DMSO or Rutin for 24h in the proportion of 1:10, 1:100, and 1:1000. DAB staining was performed to mark βIII-Tubulin. The total number of neurons was counted in an inverted microscope. **RESULTS:** We observed Aba CM decrease the motoneuron viability (31±7, $p>0.005$) when compared with control. Treatment of Aba cells with Rutin (20 micromol) impaired the Aba CM toxic effect in motoneurons viability (80±9, $p>0.05$). **Conclusion:** This result suggests that rutin decrease the production and/or release of neurotoxic factors in Aba cells. Immunocytochemistry showed that S100b was not different in Aba cells treated with Rutin. Nonetheless Ki67 index was higher in untreated Abas when compared to Rutin treatment, which may indicate a lower proliferative actitivity.. Our next step will be to characterize levels of neurotoxic cytokines in the Aba CM cells, and to correlate with neurotoxic/ neuroprotective effect of rutin.

Support: CAPES, FAPESB.

Key Words: Amyotrophic Lateral Sclerosis, Phytochemicals, Neuroprotection

ERK AND AKT SIGNALING PATHWAYS IN THE PROTECTIVE EFFECT OF DICHLOROMETHANE EXTRACT AND COUMARINS FROM AMBURANA CEARENSIS SEEDS

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INTRODUCTION: Glutamatergic excitotoxicity is one of the pathophysiological mechanisms present in chronic and acute neurodegenerative disorders, such as ischemic stroke. Excess glutamate promotes apoptosis in neurons by increasing the entry of Ca^{2+} into the cell, resulting in DNA damage and cell death. On the other hand, astrocytes actively control the glutamate excess to prevent neuronal death. Our previous studies recently demonstrated the improvement of astrocytic function by compounds present in in the diclorometh ane extract of Amburana cearensis seeds (EDAC) as the potential mechanism involved in the neuroprotective action of EDAC against oxygen-glucose deprivation. However, more studies need to be done to characterize coumarin, the main compound in EDAC, as a pharmacologically active agent. It is known that cerebral ischemia can lead to the activation of cascades such as PI3K/AKT and MAPK, increasing the expression of proteins such as AKT and ERK1/2, and modulating the expression of glutamine synthetase (GS), an astrogliosis marker, which converts glutamate to glutamine, a non-toxic amino acid. **OBJECTIVE:** Therefore, this study aims to investigate the effects of EDAC and coumarin in the regulation of astrogliosis markers and proteins of PI3K/AKT and MAPK pathway in an acute ischemic stroke model. **MATERIAL AND METHODS:** Thus, PC12 cell lines and primary cell culture of astrocytes from Wistar rats were subjected to glutamate (20 mM) and/ or treated concomitantly with EDAC (500 µg/mL) and coumarins (500 µm/mL) for 24 hours. Subsequently, cell viability was evaluated by MTT. Additionally, the expression of AKT and MAPK pathway proteins was investigated by western blot, and the expression of caspase 3 by immunofluorescence. **RESULTS**: EDAC and coumarins were able to protect PC12 cells and primary astrocytes from the toxicity of glutamate (20 mM). They were able to upregulate the expression of AKT, ERK1/2, and phosphorylated-ERK and downregulate caspase 3. **CONCLUSION:** Our results demonstrate that EDAC and coumarin have a potential pharmacological effect in models of excitotoxicity and clarify the mechanisms of action related to the neuroprotective effect of compounds from *A. cearensis* seeds in a study model of cerebral ischemia.

Keywords: Cerebral ischemia, Amburana cearensis, coumarin, ERK/MAPK pathway

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EFFECT OF THE FLAVONOID RUTIN ON GLIAL AND NEUROGENIC MARKERS IN AN *IN VIVO* MODEL OF PARKINSON'S DISEASE INDUCED BY AMINOCHROME

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Introduction: In Parkinson's disease, various cellular and molecular changes occur, including reactive gliosis and reduced neurogenesis, which persist in two brain regions throughout adulthood - one being the subgranular zone in the hippocampus dentate gyrus. Evidence suggests that microglia and astrocytes in the M1 and A1 profiles hinder neurogenesis, while M2 and A2 cells promote it. Rutin, a flavonoid, seems to reduce microglial/astrocytic reactivity and shift M1 to M2 behavior *in vitro*. However, there are no studies demonstrating rutin's potential to stimulate neurogenesis while altering microglial and astrocytic profiles. **Objective:** To evaluate the effect of rutin on the state of glial activation and associate it to its neurogenic potential. **Methodology:** We used 24 male Wistar rats divided into four groups: negative control (CT, n=6), rutin (RUT, n=6), aminochrome (AMI, n=6), and aminochrome plus rutin (AMI+RUT, n=6). Stereotaxic surgery was performed in the striatum region, applying saline to the CT and RUT groups and aminochrome to the AMI and AMI+RUT groups. After 22 days, the rats were perfused, and their brains were processed for immunofluorescence using GFAP, S100b, DXC, IBA1, CD68, CD206, and NeuN antibodies. **Result:** The analysis of the immunophenotypic profile of astrocytes and microglia in the groups treated with rutin showed that the flavonoid is capable of generating an increase in the density of GFAP+ and S100b+ cells and this astroglial change is followed by an increase in the number of CD68+ microglia. This result was followed by a numerical decrease in DXC+ and CD206+ cells in the same group. Group AMI did not demonstrate changes in the markers tested. When evaluating the AMI+RUT group, it is possible to observe a numerical decrease in DXC+ and CD206+ cells, with an increase in CD68+ cells. The NeuN marker did not show changes in the tested groups. The aminochrome that was administered at the level of the striatum did not cause changes in the hippocampus, however the rutin, when administered orally, caused changes at the level of this anatomical region.

Keywords: Parkinson's disease; Rutin; Neurogenesis.

Support: FAPESB.

ADVANCES ON THE EFFECT OF RUTIN AGAINST AMINOCHROME NEUROTOXICITY: WHAT HAPPENS IN THE SUBSTANTIA NIGRA AFTER 21 DAYS OF TREATMENT?

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Introduction: rutin is a flavonoid with medicinal properties recognized in the literature, including neuroprotective, antioxidant and anti-inflammatory potential. Aminochrome is a compound produced in dopaminergic neurons during the neuromelanin formation with ability to induce oxidative stress, alpha-synuclein accumulation, and neuroinflammation as in PD. **Methods**: male Wistar rats (280g–330g) were divided into four groups: control (CTR), 10 mg/kg rutin (RUT), 6 nmol aminochrome (AMI), and aminochrome+rutin (AMI+RUT) to perform stereotaxis and injection of aminochrome into the striatum (ICS- CEUA 114/2016). Animals were treated with daily oral dosis of rutin for 14 and 21 days. The behavioral tests were performed on the 14th day and samples of SNpc were collected 15th and 22th day for performing immunohistochemistry (IBA1+;TH+;GFAP+;S100b+;SOX10;NQ01). **Results**: the open field test showed animals from AMI group presented a reduction in the total frequency of rearing when compared with CTR group; The plus maze test showed animals from AMI+RUT group presented increased frequency of open arm entries, when compared with animals from AMI group; and animals from RUT group presented higher time spent in the closed arm when compared with CTR group. The immunohistochemical analyzes showed that aminochrome reduced the viability of TH+ cells in AMI group when compared with CTR group in both treatment times. The number of IBA1+ cells did not change significantly between the groups, however, there was a considerable increase in microglia-neuron interactions in the AMI group compared with CTR and AMI+RUT group on the 14th day. After 21 days, the colocalized expression of GFAP+ and S100b+ was greater in the AMI group when compared to the CTR and AMI+RUT group. There was no change in the number of cells that expressed NQ01 or SOX10. **Conclusions:** this study contributes to clarify a new study model of PD and the neuroprotective potential of rutin.

Keywords: Bahavioral changes, aminochrome, neuroprotection, rutin.

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CYTOKINE PROFILE AFTER ANXIETY THERAPY: A SYSTEMATIC REVIEW

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Introduction: Neuropsychiatric disorders account for a quarter of the health loss due to disability in the world population. Meanwhile, anxiety disorders are the second most prevalent class of disorders in the world. Among the others, especially Generalized Anxiety Disorder (GAD) has a chronic duration, manifested through the feeling of excessive and uncontrollable worry. The main therapeutic line is the use of selective serotonin reuptake inhibitors (SSRIs), capable of promoting changes in the chronic inflammatory state caused by generalized anxiety disorder (GAD). Furthermore, it is important to understand the neuroimmunomodulation that occurs in the cytokine level after SSRI therapy. **Objectives:** Systematically review of the cytokine profile in patients with generalized anxiety disorder (GAD) treated with selective serotonin reuptake inhibitors (SSRIs). **Methods:** It was conducted according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta Analyzes – PRISMA. The search strategy for eligible studies was carried out in the Medical Literature Analysis and Retrieval System Online (Pubmed/MedLine) and Excerpta Medica dataBASE (EMBASE) databases through subject descriptors. **Results:** Of 243 articles identified, 6 were included. The level of the pro-inflammatory cytokine IL-6 was significantly reduced in the short term after therapy with fluoxetine, setraline and escitalopram. However, IL-6 demonstrates a long-term increase after therapy with fluoxetine or escilatropam. The same was observed for the cytokine IL-1β. As for TNF-α, there was a significant reduction after therapy with fluoxetine for eight weeks and no significant change was seen within six months. IFN-γ levels did not demonstrate a significant difference in short-term treatment with escitalopram and escitalopram and setraline. Regarding the profile of anti-inflammatory cytokines, IL-2 and IL-10 did not demonstrate a significant difference in SSRI therapy, which has not been reported in the long term. **Conclusion:** The anti-inflammatory potential of SSRIs is related to the short period of use, with a significant reduction in IL-6, IL-1β and TNF-α. In the long term therapy, it is suggested that SSRIs do not interfere with the pro-inflammatory cytokines IL-6 and IL-1β. In addition, there was not significant changes in relation to the anti-inflammatory cytokines IL-2 and IL-10 in the short and long term therapy.

Keywords: Selective serotonin reuptake inhibitors; generalized anxiety disorders, immunomodulation.

ANALYSIS OF THE EFFECTS AND MECHANISMS OF ACTION OF FLAVONOIDS IN CONTROLLING NEUROINFLAMMATION IN PRECLINICAL MODELS AND IN CELLS FROM PATIENTS WITH MULTIPLE SCLEROSIS

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Introduction: Multiple sclerosis is an autoimmune inflammatory disease of the CNS of unknown etiology, characterized by demyelination and variable degrees of axonal loss. The immunopathogenesis of MS is believed to involve a violation of self-tolerance to myelin and other CNS antigens, resulting in persistent peripheral activation of autoreactive T cells. Tryptophan catabolites, through the so-called kynurenine pathway, have shown a relevant role as immunomodulatory and neuroactive intermediates, and have been linked to the pathophysiology and prognosis of neurodegenerative diseases. Agathisflavone (bis-apigenin) is flavonoid extratec from *Caesalpinia pyramidalis* leaves, has demonstrated neuroprotective effects, associated with anti-inflammatory, antioxidant and myelinogenic actions in cortical and cerebellar cultures. **Objective:** This study will evaluate the effects and mechanisms of action of agathisflavone and its monomer apigenin in the control of neuroinflammation in preclinical models and in monocytic cells from patients with MS. **Methods:** Mononuclear cells will be obtained from the blood samples from healthy donors and from MS patients. Cell cultures will be performed using organotypic and dissociated cultures of cerebellar cells from postnatal mice. The inflammatory profile will characterized by immunocytochemistry for inflammatory and regulatory markers of microglia/macrophages and astrocytes. Metabolites from the kynurenine pathway will be determined in the serum and in the medium of cultures by HPLC. Furthermore, to evaluate the anti-inflammatory action of polyphenols, expression of kynurenine enzymes, cytokines, neurotrophic factors and markers of glial activation, the RT-qPCR technique will be used. Finally, the ELISA method will be used to evaluate markers of neuroinflammation. **Expected Results:** To characterize the effects and mechanisms of action of polyphenols agathisflavone and apigenin in the control of neuroinflammation and myelination in in vivo and in vitro study models of MS, and association with modulation of production of catabolites of the kynurenine pathway.

Keywords: Multiple sclerosis. Neuroinflammation. Agathisflavone

Support: FAPESB, INCT-Translational Neuroscience, CAPES.

AGATHISFLAVONE RELIES ON GLUCOCORTICOID RECEPTOR TO EXERT ITS REGULATORY EFFECTS ON ASTROCYTES AND MICROGLIA *IN VITRO*

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Neuroinflammation is implicated in the pathophysiology of neurodegenerative diseases. Nuclear receptors, such as glucocorticoid receptors (GR), are transcription factors with multiple effects, prominently regulatory effects on neuroinflammation. GR is one of the main regulatory transcription factors and is highly expressed in the central nervous system. Agathisflavone (bis-apigenin), is a phytoestrogen with demonstrated neurogenic, neuroprotective, anti-neuroinflammatory, antioxidant, and pro-myelinogenic effects in *vitro*. These studies demonstrate agathisflavone's dependence on estrogen receptor (ER) activity to exert its aforementioned regulatory effects. However, agathisflavone's mechanisms need further elucidation. The objective of this study was to investigate whether the regulatory action of agathisflavone is mediated by GR. For this, an *in vitro* model of neuroinflammation induced by lipopolysaccharide (LPS) in the primary culture of astrocytes and microglia obtained from the cerebral cortex of neonatal rats was used. Cells were exposed to LPS (1 µg/mL), associated or not with agathisflavone (1 µM), and in the presence or not of mifepristone (RU486; 1 µM), a GR antagonist. Microglial inflammatory profile was evaluated by their morphology through immunofluorescence against calcium-binding ionized adapter molecule (Iba-1), and for CD68, a marker of microglial pro-inflammatory profile. The astrocytic inflammatory profile was evaluated by immunofluorescence against glial fibrillary acidic protein (GFAP). Microglial branching was increased in response to treatment with agathisflavone, an effect inhibited in the presence of mifepristone. CD68 expression was decreased by agathisflavone, but not in the presence of mifepristone. The relative GFAP expression was decreased by agathisflavone, but not in the presence of mifepristone. These results suggest that agathisflavone attenuates microglial and astrocytic proinflammatory profiles via GR activity during inflammatory stimulus. These results contribute to elucidating the molecular mechanisms of agathisflavone in the nervous system.

Keywords: neuroinflammation, glucocorticoid receptor, microglia, astrocytes, agathisflavone, neuroprotection.

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INVOLVEMENT OF TRYPTOPHAN METABOLISM IN THE ANTI-NEUROINFLAMMATORY ACTIVITY OF THE FLAVONOIDS AGATHISFLAVONE (APIGENIN AND BIS-APIGENIN)

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Introduction: Tryptophan (Trp/W) is an essential amino acid important for the synthesis of proteins and neurotransmitters such as serotonin and melatonin. Trp is bound to serum albumin and the remainder is freely available in the blood and is able to cross the blood-brain barrier and can be transformed into bioactive molecules by CNS cells. In inflammatory processes, cells such as macrophages, astrocytes and microglia produce indoleamine 2, 3-dioxygenase (IDO), enzyme responsible for the transformation of Trp into kynurenines (KYN). KYN can undergo the action of kynuriniases, and the enzyme kynurenine 3-monoxygenase (KMO) produces quinolinic acid (QUIN), important in the formation of nicotinamide, a component of the NAD+ hydrogen transport. Metabolites of this pathway, such as QUIN and kynurenic acid (KINA), can modulate the expression of several cytokines, chemokines and their receptors in astrocytes, with an important role in neuroinflammation. The flavonoid agathisflavone (bis-apigenin), has demonstrated neurogenic, neuroprotective and anti-neuroinflammatory properties, however mechanism of action are still few elucidated **Objectives:** This work investigated the possible role of Trp pathway in the agathisflavone effects in an in vitro model of neuroinflammation. **Methods** Isolated microglia and mixed glial (astrocytes and microglia) primary cultures obtained from newborn rats (P2) were modulated with agathisflavone (1 µM) in the presence or not of LPS (1 µg/mL), QUIN (500 nM), or with the IDO inhibitor, 1-methyl tryptophan. It was observed that LPS and QUIN induced toxicity, evidenced by the MTT test, and morphological changes in glial cells, evidenced by immunocytochemistry, characterizing gliosis, effects also observed in cultures modulated with IDO inhibitor, and reversed in cultures also treated with agathisflavone. **Results and Conclusions** Components of the tryptophan pathway may be associated with anti-neuroinflammatory effects of the flavonoid agathisflavone.

Keywords: Tryptophan; kynurenines; agathisflavone (bis-apigenin)

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