University of São Paulo

School of Pharmaceutical Sciences of Ribeirão Preto

Graduate Program in Biosciences Applied to Pharmacy

Abstract collection

I WORKSHOP OF BIOSCIENCES AND BIOTECHNOLOGY

Organization:

Graduate Program Coordinator: Profa. Dra. Andréia Machado Leopoldino

Graduate Program Vice-Coordinator: Profa. Dra. Ana Patrícia Yatsuda Natsui

Members of the Graduate Program Coordination Committee:

Profa. Dra. Fabiani Gai Fantz

Prof Dr José Clóvis do Prado Junior

Profa. Dra. Elisa Maria de Sousa Russo

Profa. Dra. Ana Amélia Carraro Abrahão

Profa. Dra. Kelen Cristina Ribeiro Malmegrim de Farias

Profa. Dra. Maria Regina Torqueti

Secretary:

Henrique Theodoro

Postdoctoral Fellows:

Dra. Glauciane Garcia de Figueiredo

Dra. Larissa Pernomian

Dr. Leonardo Neves de Andrade

Dr. Luiz Gustavo Gardinassi

Dr. Luiz Miguel Pereira

Dra. Vânia Brazão Muniz

Dra. Vânia Santos Braz

December 2017

Acknowledgements

The organizing committee deeply appreciate the financial support provided by the funding agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) to professors and students of the Graduate Program in Biosciences Applied to Pharmacy. We also especially thank the financial support provided by **Sil life Biotecnologia**, SP, Brazil.

P01 - RECOMBINANT ANTIBODY FRAGMENTS AGAINST SYNTHETIC GLYCOPEPTIDE MIMICKING CANCER MUCINS: A PROMISING TOOL FOR DIAGNOSIS AND DRUG DELIVERY SYSTEMS.

Thais Canassa De Leo[1], Marcelo M. Brígido[2], Andréa Q. Maranhão[2], Vanessa L. Campo[1], Marcelo Dias-Baruffi[1]

1 School of Pharmaceutical Sciences of Ribeirao Preto, University of Sao Paulo, Brazil

2 Institute of Biological Sciences, University of Brasilia, Brazil.

Cancer is a severe disease associated with alterations in the health molecular glycosylation patterns that lead to pathophysiologic consequences and to the discovery of cancer-biomarkers. Hypoglycosylated MUC1 (transmembrane glycoprotein Mucin 1) is an important tumoral marker for several types of cancer. Two of the most important challenges in cancer clinical research are the development of precise diagnostic and therapeutic strategies that allow the targeting of anti-tumor drugs direct to the cancer cell and not to healthy cells. Thus, the development of site-specific drug delivery and diagnostic systems based on tumoral markers are important approaches in the fight against cancer. Here we described DNA sequence, cloning, and expression of selected human recombinant antibody fragments against synthetic glycopeptides based on MUC1. Accordingly, the synthetic glycopeptide NHAcSer-Ala-Pro-Asp-Thr[βGalNAc]-Arg-Pro-Ala-Pro-Gly-BSA, mimetic of hypoglycosylated MUC1, was obtained by solid phase synthesis followed by conjugation reaction with the carrier protein BSA. This conjugated glycopeptide was then immobilized on ELISA microplates for selection of antibodies fragments from a human antibody library, by Phage Display. Coding genes of selected antibodies were sequenced in Illumina Miseq system and analyzed using a novel platform (ATTILA). The results indicated that Phage Display selection for anti-synthetic glycopeptides was very efficient, since the last 4th round was enriched around 10,000-fold relative to initial round. Also, the most enriched sequences were associated with the higher affinity to tumor-associated antigen. The best two sequences were cloned into a pET29(a) expression vector. Expression of a recombinant anti-MUC1 scFv was performed on E. coli BL21-DE3 and purification was performed by affinity chromatography on nickel resin, followed by gel filtration, these steps being monitored by SDS-PAGE. Immunochemical identification of the recombinant protein was confirmed by Western Blot. We further propose to use the soluble anti-MUC1 glycopeptide antibody fragments to develop novel chemotherapy delivery and cancer cell detection systems.

Keywords: MUC1, Glycopeptide, Phage Display, Antibody, Cancer

Financial Support - Capes, FAPESP.

P02 - Identification and antifungal susceptibility of Aspergillus spp. in clinical settings

BRAGA, V. F.[a]; BARIÃO, P. H. G.[a]; TONANI, L.[a]; NASCIMENTO, E.[b]; MARTINEZ, R.[b]; VON ZESKA KRESS, M. R.[a]

[a] Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, 14040-903 Brazil;

[b] Departamentode Clínica médica, área de moléstias infecciosas, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, 14040-903 Brazil.

Aims. The correct identification of the species and the determination of the antifungal susceptibility will aid to better understand the evolution of invasive and non- invasive aspergillosis. Methods. Genomic DNA from these isolates was extracted and used for the molecular identification by PCR amplification of rDNA ITS region and the gene that encodes the calmodulin. The sequences analyzed in ChromasPro program and the polymorphism study was performed using the Molecular Evolutionary Genetics Analysis program-MEGA 6.0 by the method of multiple alignments. The identification of isolates was carried out by agreement of the results obtained in the analysis of morphology (macro and micro) and molecular analysis. The antifungal susceptibility was tested for clinical isolates according to the Protocol M38A of the Clinical and Laboratory Standards Institute (CLSI). Antifungals of the class of polyenes (amphotericin B) and azole (itraconazole and voriconazole) were tested. Results. The molecular identification has shown that out of a total of 23 isolates, 9 belong to the section Flavi and 11 belong to the section Fumigati. Within the section Flavi, a geometric mean (MG) of the minimum inhibitory concentration (MIC) of 1.25 µg/mL to amphotericin B, 0.73 µg/mL to itraconazole and 1.85 µg/mL to voriconazole were observed. The isolates belonging to the section Fumigati have shown the values of GM-MIC of 0.88 µg/mL,1.06 µg/mL and 2.13 µg/mL to amphotericin B, itraconazole and voriconazole, respectively. Conclusions. In conclusion, species belonging to the Flavi and Fumigati sections have been isolated from clinical specimens in which all isolates are sensitive to antifungal agents, except for three isolates from the Fumigati section (identified as A. fumigatus) that demonstrated high values of minimum inhibitory concentration.

Keywords. Aspergillus, section Fumigati, section Flavi, molecular identification, in vitro susceptibility

Financial Support. CNPq, CAPES, FAPESP

P03 - Development of real time RT-PCR for the differential diagnosis of chikungunya, dengue and Zika.

Lippi, Veronica[1], Trabuco, AC[1], Figueiredo, LT[2], Coelho, HC[1], Aquino, VH[1].

1 FCFRP-USP, Ribeirão Preto, Brazil

2 FMRP-USP, Ribeirão Preto, Brazil

Chikununya (CHKV) and Zika (ZIKV) viruses are transmitted by the bite of female mosquitoes of Aedes aegypti specie, the same mosquito that transmits the dengue virus (DENV). The CHIKV is associated with a rheumatic disease, which in some cases can become chronic, while ZIKV has been associated with several neurological manifestations, with microcephaly in newborns been the more severe form. DENV can cause a self-limiting febrile illness, but in some cases the disease can be more severe with hypovolemic shock that can lead to death. However, initial clinical manifestations caused by the infection with any of the three viruses are very similar, making difficult the differential clinical diagnosis. The real time RT-PCR are becoming the standard method for early diagnosis of these viral infections. Although there are some real time RT-PCR methods available commercially for CHIKV, DENV and ZIKV, the sensitivity and specificity of them should be assessed. The objective of this work is to develop real time RT-PCR methods for early diagnosis of chikungunya, dengue and Zika. Primers and probes specific for the four DENV serotypes, CHIKV and ZIKV were selected from highly conserved regions using the CLC Genomics Workbench (QIAGEN, USA). The laboratory strains Mochizuki (DENV-1), NGC (DENV-2), BR/SL3/02 (DENV-3), H87 (DENV-3), H241 (DENV-4), patient (ZIKV) and S27AS (CHIKV) were used in this study. The real time RT-PCR for each virus was standardized analyzing different primers, probes and Mg+ concentrations, in addition to the times and temperatures of the amplification cycle. Ten-fold serial dilutions of the viral RNAs were analyzed with the standardized real time RT-PCR, showing that the lower limit of detection of the tests were 104 copies/mL for the four serotypes of DENV, 104 PFU/mL for ZIKV and 102 PFU/mL for CHIKV. These tests showed to be sensitive enough to detect the viruses in clinical samples.

Keywords: Virus, Dengue, Zika, Chikungunya, Flavivirus, Arboviruses, Real Time RT-

PCR, Diagnosis.

P04 - Evaluation of the role of galectin-1 and galectin-4 on experimental infection by *Leishmania (Leishmania) amazonensis*

Anna K A Fleuri[1], Thalita B Riul[1], Rodrigo P P Soares[2], Sean R Stowell[3], Richard D Cummings[4] and Marcelo Dias-Baruffi[1]

1 School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil. 2 Centro de Pesquisas René Rachou/FIOCRUZ, Belo Horizonte, Minas Gerais, Brazil. 3 Emory University School of Medicine, Atlanta, GA-USA, 4 Harvard Medical School, Boston, MA 02115, USA.

Leishmaniasis is a disease caused by infection of Leishmania genus parasites and it represents a major public health concern in many countries of Africa, Asia and Latin America. Studies have shown that the resolution of this infection is dependent of homeostatic immune response from the host. Nevertheless, there are still many gaps in knowledge related to its pathogenesis. The participation of galectins, proteins that recognize glycans with beta-galactoside motifs, has been described in several infectious diseases, although there are few reports on the involvement of galectins in Leishmaniasis. We aim to evaluate the role of galectin-1 (Gal-1) and Gal-4 in experimental infection by Leishmania sp. We have demonstrated that Gal-1 deficiency in BALB/c mice (Lgals1-/--BALB/c), but not in Lgals1-/--C57BL/6, promotes restriction of L. amazonensis (La) infection. This outcome was related to increased IFN-γ production and decreased IL-4, IL-10, IL-12p70, and TNF-α and decreased lesion size and parasite load at the infection site. Gal-1 did not bind to this parasite, suggesting that the susceptibility of WT BALB/c was not due to a direct interaction between Gal-1 and Leishmania. Full length Gal-4, and not their C- and N-terminal domains, recognizes La in dose and carbohydrate-dependent manners. The glycoconjugate lipophosphoglycan (LPG) from these parasites is a molecular target for full length Gal-4. These results demonstrate that galectins can participate in the immune response against Leishmania and open new avenues for therapeutic and/or diagnostic strategies applicable to this neglected disease.

Financial support: CAPES and CNPq

P05 - Metagenomic approach based on 16S rRNA gene for evaluation of bacterial population of Brazilian milk and dairy products

Diego A. Frazílio[1], Fabian C. N. Arias[1], Vanessa M. de Souza[1], Luiza T. Chaul[2], Virgínia Farias Alves[2], Elaine C.P. De Martinis[1]*

1 Faculdade de Ciências Farmacêuticas de Ribeirão Preto (FCFRP) – USP; 2 Faculdade de Famácia – Universidade Federal de Goiás, Brazil. Corresponding author: edemarti@usp.br

INTRODUCTION: Bacterial populations in milk and dairy products are of great interest from a food safety point of view and also due to the presence of cultures with potential technological applications. To evaluate the main bacterial groups present in Brazilian dairy products, metagenomic analysis targeting the 16S rRNA gene was done, using the platform Illumina® Miseq. Dairy plants from Brazilian midwest region were sampled for raw material, food contact surfaces, food non-contact surfaces and ready to eat products, in a total of 12 representative samples. Total DNA was extracted directly from the samples using the commercial kit PowerLyzer® PowerSoil® DNA isolation (MoBio®), according to instructions of the manufacturer and, DNA libraries were prepared according to the protocol of Illumina®. DNA sequencing was done in the equipment Illumina® MiSeq, at the FCFRP-USP and analyzes were done with the dedicated software available in the sequencer. RESULTS: The most prevalent species in the dairy samples were Enterococcus casseliflavus (28%), Enterococcus faecium (6%), Lactococcus lactis (6%), Staphylococcus agnetis (4%), Staphylococcus hiyicus (4%). Staphylococcus aureus was detected only in raw material and other pathogens were not found in the samples evaluated.

P06 - IN VITRO EVALUATION OF CYTOTOXICITY OF GENISTEIN ISOLATED AND COMBINED WITH TAMOXIFEN IN BREAST CANCER CELL LINES

Patrícia Heloise Alves Bezerra(1), Maria Regina Torqueti(1)

1. University of Sao Paulo, Pharmaceutical Sciences College of Ribeirao Preto

Abstract: Breast cancer is the neoplasm with the most number of cases in women and the second one that most kill worldwide. Tamoxifen is an example of SERM (selective estrogen receptor modulator) that is used in breast cancer therapy since it presents anti-estrogenic and anti-oncogenic properties. However, besides the side effects, it is common cases of patients who develop resistance to the Tamoxifen treatments. Literature indicates phytoestrogens from food as a protective factor against breast cancer in asian women through the consumption by the soy and derivatives. Thus, the objectives of this study was to evaluate the cytotoxicity of Genistein isolated and combined with Tamoxifen against estrogen-dependent (MCF-7), estrogen-independent (MDA-MB-231), and overexpressing HER-2 (SKBR-3) breast cancer cell lines and the control cell line (MCF-10A), using the method of neutral red uptake. After plating and treating cells, neutral red is added and viable cells can accumulate it inside lysosomes, this way it is possible to differentiate viable from non-viable ones through spectrophotometry. Therefore, when isolated Genistein has provoked an accentuated cytotoxicity on the three types of breast cancer cell lines. Such cytotoxicity was observed also when the phytoestrogen was combined with the most abundant metabolite of Tamoxifen. However, low doses of this isoflavone must be administered with caution, since they may enhance the tumoral proliferation when associated with the other metabolite of Tamoxifen.

Key words: breast cancer, Tamoxifen, Genistein.

P07 - MOLECULAR IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY OF CRYPTOCOCCUS GATTII CLINICAL ISOLATES

BARIAO, P.H.G.; BRAGA, V.F.; NASCIMENTO, E.; TONANI, L.; MARTINEZ, R.; VON ZESKA KRESS, M.R.

The Cryptococcus spp. are encapsulated yeast-like fungi that have two main species pathogenic to humans C. neoformans that affects immunocompromised patients and C. gattii that affects immunocompetent persons. The aim of this study was to analyze and evaluate the fungal clinical isolates of patients with cryptococcosis from Hospital das Clinicas da Faculdade de Medicina de Ribeirão Preto (HCFMRP) – USP between 2013 and 2017. To this, Cryptococcus spp. clinical isolates were identified by molecular methods and the susceptibility to commercial antifungal agents (amphotericin B, voriconazole and fluconazole) were determine by in vitro assay. The molecular identification of these species were performed by convetional PCR with 2 pairs of specific primers to differentiate C. neoformans (CNa-70A and CNa-70S) and C. gattii (CNb-49A and CNb-49S). The clinical isolates identified as C. gattii were submitted to in vitro antifungal susceptibility test evaluation by the broth microdilution method based on the MS27-A3 protocol from the Clinical and Laboratory Standard Institute (CLSI). In a total of 132 Cryptococcus spp. isolated from different anatomical sites, 18 (13.6%) were molecularly identified as C. gattii and 114 (86.4%) were C. neoformans. Results of minimum inhibitory concentrations (MIC) of C. gattii isolates were presented by geometric mean (GM) and MIC-range, respectively, for amphotericin B 0.65 ug / mL (0.50 - 1.0 ug / mL), fluconazole 1.27 ug / mL (0.06 - 4.0 ug / mL) and voriconazole 0.29 ug / mL (0.06 - 1.0 ug / mL). All C. gattii isolates showed sensitivity to the antifungal agents used.

Keywords: Cryptococcus gattii; molecular identification; antifungal susceptibility.

Financial Support: CAPES, CNPq e FAPESP

P08 - DETERMINATION OF THE THERAPEUTIC POTENTIAL OF ASCORBIC ACID IN EXPERIMENTAL CHAGAS DISEASE: AN AUXILIARY TREATMENT

Maiara Voltarelli Providello[1]; Zumira Aparecida Carneiro[1]; Gabriel Tavares do Vale[2]; Gisele Bulhões Portapilla[1]; Ricardo Souza Camargo[3]; Carlos Renato Tirapelli[4]; Sérgio de Albuquerque[1]

1 Department of Clinical, Toxicological and Bromatological Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil

2 Department of Pharmacology, School of Medicine, University of Sao Paulo (USP), Ribeirão Preto, SP, Brazil

3 Department of Biochemistry and Immunology, School of Medicine of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil

4 Department of Psychiatric Nursing and Human Sciences, Ribeirão Preto School of Nursing, University of São Paulo (USP), Ribeirão Preto, Brazil

Nowadays seven million people are infected with Trypanosoma cruzi worldwide. Benznidazole (BZ) is the only treatment available in the country, it has low efficacy and serious side effects. Wherefore the greatest challenge for researchers is the development of new therapies. In addition, Chagas disease involves an intense inflammatory process that is strictly linked to the establishment of oxidative damage, making the process of tissue destruction even more severe. Therefore, studies using antioxidants in disease therapy have been gaining the attention of scientists. In the present research, ascorbic acid (AA), an important antioxidant, was tested alone and in combination with BZ in a subclinical dose (10mg / kg). Our aim was to evaluate if the substance had a trypanocidal effect and if it could bring benefits to the current therapy in relation to oxidative stress. Male Swiss mice experimentally infected with Trypanosoma cruzi strain Y were treated orally during fifteen days. The results obtained suggest that the association between the substances (AA+BZ10) presented synergy in the reduction of the number of circulating parasites. Besides, a decrease in the levels of intracellular reactive oxygen species (ROS) was observed as well as lipoperoxidation in cardiac tissue in the mentioned group, evidenced by the dosage of thiobarbituric acid reactive substances (TBARS). It was noted that the antioxidant alone or in association with BZ significantly decreased the parasitism in the heart detected by qPCR, however histological analyzes revealed a greater reduction in the inflammatory infiltrate in this organ when the substances were administered concomitantly. Thereby, it is concluded that AA could bring benefit to the treatment of this pathology if associated with reduced doses of BZ. Oxidative parameters reveal that, in the long term, preservation of cardiac tissue should occur.

Key-Words: Ascorbic acid; Chagas disease; Oxidative stress; Reactive oxygen species.

P09 - Expression of Mitochondrial NAD+ Transporter from Aspergillus fumigatus Recovers the Metabolism in HEK 293 Cells with Citrin Gene Knockdown

Laís de Lourdes de Lima Balico[1], Emerson de Souza Santos[1], Silveli Suzuki-Hatano[2], Lucas Oliveira Sousa[1] and Sérgio Akira Uyemura[1]

1 Department of Clinical, Toxicology and Bromatology Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo.

2 Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of

Type 2 citrullinemia is a mitochondrial disease mainly caused by citrin protein deficiency. This disease can cause cytosolic NADH/NAD+ ratio increase and alters glucose metabolism. Aspergillus fumigatus has a mitochondrial NAD+ transporter (Ndt1 protein), which catalyzes transport of cytosolic NAD+ to mitochondrial matrix. Heterologous expression of transkingdom gene has recently emerged as an alternative for recovery of human mitochondrial diseases. The aim of this study was to characterize HEK 293 with citrin gene (SLC25A13) knockdown expressing Ndt1 protein from A. fumigatus in comparison to HEK 293 cells. SLC25A13 sh1RNA was purchased from Sigma. After cell transduction, stable cells were selected with puromycin. Transduction was confirmed by qRT-PCR. qRT-PCR showed reduction of citrin gene expression in 44%. The pcDNA/ndt1 construction was transfected into HEK 293 cells and stable line was selected with geneticin. Ndt1 expression and SLC25A13 gene knockdown were confirmed by western blot and Ndt1 protein co-located in mitochondria. Total cell and mitochondrial concentrations of NADH and NAD+ were determined using NAD/NADH assay kit. Ndt1 protein and citrin gene knockdown alters total cell and mitochondria concentrations of NADH, NAD+ and NADH/NAD+ ratio. However, Ndt1 expression reduced the increase of these cofactors caused by citrin deficiency. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were determined in SeaHorse XF24 Analyzer. The citrin deficiency altered the OCR and ECAR probably due to a higher NADH/NAD+ ratio in these cells. In citrin deficient cells, Ndt1 expression decreases NADH/NAD+ ratio and recovers OCR. Nonetheless, these cells showed an increase in lactate concentration as well as in basal and maximal ECAR. These results suggest that Ndt1 expression recovers metabolism in citrin deficient cells.

Keywords: Citrullinemia, mitochondria, metabolism, Ndt1, fungi.

Financial Support: CNPq, CAPES, FAPESP

P10 - STUDIES ON SPHINGOSINE KINASES IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

FUGIO, L.B.[1]; BRAUER, V.S.[1]; FIGUEIREDO, L.S.[1]; SANTOS, T.M.[1]; CURTI, C.[1]; LEOPOLDINO, A.M.[1]

Head and neck squamous cell carcinoma (HNSCC) can arise from oral and nasal cavity, salivary glands, pharynx, larynx, and paranasal sinuses. HNSCC is the sixth most common type of cancer and eighth cause of death worldwide. Several studies have suggested alterations of sphingolipids in cancer. These “bioactive lipids” are a class of membrane lipids essential in cell structure and currently stand out as key regulators in different cellular processes. The main sphingolipids are ceramide and sphingosine, that mediate apoptosis regulation and cellular senescence, and sphingosine-1-phosphate (S1P), that plays a crucial role in cell survival and migration. The balance between phosphor- and unphospho-sphingosine can be regulated by sphingosine kinases (SphK1 and SphK2). While SphK1 has been implicated in tumor growth and cell transformation in HNSCC, the role of SphK2 in HNSCC is unknown. Therefore, our goal is to understand how SphK1, SphK2 and sphingolipids participate in HNSCC, looking for potential therapeutic targets and strategies in cancer. The cellular levels of the SphKs in HNSCC tissue samples were determined by immunohistochemistry, and in tumor and non-tumor cell lineages by Western blot (WB). Sphingolipids profile was assessed by mass spectrometry, cellular distribution of proteins by immunofluorescence and mRNA levels by real-time PCR (qRT-PCR). Knockdown of target proteins was performed by interference RNA, overexpression of SphKs proteins in cell lineages by using vectors with the cDNAs, and cell cycle analysis by propidium iodide staining/flow cytometric. A panel of HNSCC cell lineages were analyzed and important alterations in SphKs were observed, suggesting a deregulation in sphingolipids balance in these cells. Overexpression using DNA constructs of SphKs in a non-tumor keratinocyte cell lineage was confirmed by WB and qRT-PCR. Colony formation assay showed different ability for clonogenicity potential, suggesting a role of SphKs in HNSCC. Besides, alterations in signaling pathways were observed when SphKs were overexpressed in non-tumor keratinocyte cells, affecting cellular parameters such as proliferation and cell cycle distribution. These results indicate that SphK2 is at least as important as SphK1 in HNSCC.

Keywords: head and neck cancer, sphingolipids, sphingosine kinase, cellular signaling.

Financial support: FAPESP, CEPID, CAPES and CNPq.

P11 - DNA methyltransferase-mediated immunomodulatory effects in monocytes infected by Mycobacterium tuberculosis

Zambuzi FA[1]; Cardoso-Silva PM[1]; Castro RC[1]; Galvão-Lima LJ[1]; Fontanari C[1]; Emery FS[1]; Frantz FG[1]

1. Faculdade de Ciências Farmacêuticas de Ribeirão Preto – FCFRP-USP, Ribeirão Preto, São Paulo, Brazil

Epigenetic modifications may result in gene expression changes, which are related to the pathogenesis of various diseases. These alterations are targets of drugs, known as epi-drugs, due to their reversal potential, aiming to reestablish the gene expression pattern. Among these epi-drugs are hypomethylating agents, such as 5-aza-2-deoxycytidine (Decitabine) that act by inhibiting DNA methyltransferase (DNMT), and consequently, inhibiting DNA methylation. Currently, they are used in the treatment of Myelodysplastic Syndrome, presenting an evident advantage in the survival of patients. Although the focus is on tumor cells, the administration of the hypomethylating agents occurs systemically, and therefore, epidrugs may affect several cells of the organism, including immune cells. Epidrugs can trigger immunomodulatory effects, which reinforces the hypothesis of immunoregulation in treated patients. Thus, establishing the effects of Decitabine in cells of the immune system is critical since treated patients report increased susceptibility to infections, among them tuberculosis. In this context, the objective of the present study consist in evaluate the immunomodulatory effect of Decitabine in function of monocytes infected in vitro with Mycobacterium tuberculosis (Mtb). For this purpose, we obtained peripheral blood mononuclear cells from 14 health individuals, according to the ethical principles (CEP/FCFRP nº421 – CAAE nº 59466716.1.0000.5403). Monocytes were purified and treated in vitro with Decitabine, prior to in vitro infection. Decitabine at 5 μM, slightly increased classical monocytes subpopulation, characterized by CD14 and CD16 surface molecules expression, improved the phagocytic capacity but impaired Mtb killing, in a non-dependent way of reactive oxygen species. Treatment also upregulated TLR2 expression and NF-κB activation, which reflects the increased of chemokines production, as IL-8 and RANTES, upon Mtb challenge. Further, we suggest that DNMT may mediate immunomodulatory effects in monocytes that could be determinant during tuberculosis infection in patients potentially treated with these hypomethylating agents.

Keywords: DNMT inhibitors, immune response, Mycobacterium tuberculosis

Financial Support: Fundação de Amparo à Pesquisa do Estado de São Paulo [grant number 2017/05365-8] and Coordenação de Aperfeiçoamento de Pessoal de nível Superior (CAPES).

P12 - Visible light up-regulates genes associated with stress tolerance in the entomopathogenic fungus Metarhizium acridum

Guilherme T. P. Brancini, Márcia Eliana da S. Ferreira, Gilberto Ú. L. Braga

Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo.

Aims. Metarhizium acridum is an important entomopathogenic fungus currently used for the biological control of acridids. Success of this approach is dependent on fungal survival under field stresses including ultraviolet exposure and elevated temperatures. We have previously reported that exposing mycelium to visible light induces increased tolerance to ultraviolet-B radiation. Here we employed mRNA-Sequencing to investigate gene regulation by visible light and gain further insight into the light-induced tolerance to ultraviolet radiation.

Methods. Conidia were scrapped from cultures, inoculated in liquid medium and allowed to develop for 24 h to produce mycelium. Then, mycelium was exposed to visible light for 5 min and incubated in the dark for different time periods (0, 10, 25, 55, and 115 min). A control receiving no light stimulus was also prepared. Mycelia from treatments were collected by filtration and immediately frozen in liquid nitrogen. Cells were disrupted by grinding under liquid nitrogen with mortar and pestle. Total RNA was purified with RNeasy Plant Mini Kit (Qiagen) and quality was assessed with Agilent Bioanalyzer 2100. Library construction was performed with Illumina TruSeq Stranded v4 kit and sequencing with an Illumina Hi-Seq 2500. Results were analyzed with the Hisat2-Cufflinks-Cuffdiff-cummeRbund pipeline.

Results. Our analysis encompassed 9516 genes (95.4% of the genome). Of these, 4819 genes (50.6%) were regulated by light (significance level = 0.01). A total of 2230 genes (46.3%) were up-regulated and 2589 (53.7%) were down-regulated. Cluster analysis grouped light-regulated genes into six clusters according to expression pattern. We focused our analysis on Cluster 6 which comprises 143 genes characterized by strong and quick up-regulation. In this cluster, 55 genes (38.5%) were found to be uncharacterized. Stress-related genes in this cluster include genes coding for thioredoxin reductase, heat shock proteins, UV-endonuclease, photolyase, and mismatch recognition.

Conclusion. Light quickly and strongly up-regulates stress-related genes in M. acridum.

Keywords: Metarhizium; light; ultraviolet; stress; repair

Financial Support. We thank FAPESP for a Ph.D. scholarship to GTPB and for research grants to MESF and GULB.

P13 - Isolation of Polyethylene degrading- Paenibacillus sp.

Danae Kala Rodríguez Bardají and Eliana Guedes Stehling.

Polyethylene (PE) is a thermoplastic and also a linear hydrocarbon polymer consisting of long chains of the ethylene monomers. It represents up to 64% of the synthetic plastics produced, and they are mainly used for manufacturing plastic bags, bottles and disposable containers. Then a large amount of polyethylene gets accumulated in the environment, generating plastic waste ecological problems. Microorganism's role is very important for plastic degradation. The purpose of this study was to isolate bacteria from a waste disposal area with polyethylene degrading potential. Soil samples were collected from a waste disposal region located in Ribeirão Preto, SP, Brazil to isolate bacteria. The soil samples were collected at a depth of 3-5cm, in a sterile container and then air dried at room temperature. From each soil sample collected, 1 g was added to a 10 mL BHI tube. After 24 hours of incubation at 37 ° C, 10 ml of the culture were inoculated in Erlenmeyer flask with 90 mL of MSM (minimal salt medium) containing polyethylene bags, cut into small 5 cm diameter disks. Then 200 µL were inoculated in BHI plates and plastic pieces transferred to fresh MSM along with the culture. After getting the isolates PCR reactions were performed to detect the alkB gene, associated with polyethylene degradation. Bacteria that showed the alkB gene in their genome were identified and used for degradation tests. Five bacteria were isolated but only one showed the alkb gene. This bacterium was identified as Paenibacillus sp. using the 16S rDNA gene sequencing. Used bags were heat or chemically treated with tween 80, bleach and ethanol solution and then incubated with Paenibacillus sp. for 3 months. After incubation with plastic bags and MSM, Fourier transform infra-red (FT-IR) analysis was performed. The structural change in the LDPE surface was investigated using the EQUINOX 55 FT-IR spectrometer. FTIR showed polyethylene degradation. When treated with heat it could be noted there was a slight variation in the intensity of bands in different regions compared to control when test samples (after incubation with Paenibacillus sp.) were analyzed. Changes in the peak values of almost all functional groups were observed with chemically treated bags supporting the conformational change on polymer surface and the polymer degradation. Our results indicated a great degradation potential of Paenibacillus sp. isolated from a waste disposal area. Further research will be done to support this approach.

Funding: CAPES, FAPESP

P14 - Evaluation of the Trypanosoma cruzi Mucin's Synthetic Glycopeptides Potential Protective Activity and its Antibodies in Experimental Infection

Silva, T. S.[1]; Riul, T. B[1].; Campos, V. L[1].; Albuquerque, S[1].; Baruffi, M. D.[1]

1 Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, Av. Café S/N, CEP 14040-903, Ribeirão Preto, SP

The etiological agent of Chagas disease is the intracellular protozoan Trypanosoma cruzi (T.cruzi). This disease is endemic in South America and presents a global epidemiological profile, being 10 million people around the world affected by this disease. This parasitosis presents high morbidity and can be fatal. At the present moment, there is no vaccine for this disease and the treatment strategies and diagnostic approaches are not fully efficient for coping this important parasitic infection. The goal of this work is to contribute to the development of new applicable therapeutic/diagnostic strategies for the Chagas disease from the assessment of the Trypanosoma cruzi mucin's synthetic glycopeptides potential protective activity and its antibodies in experimental infection. Murine polyclonal and monoclonal antibodies for these glycopeptides will be obtained. The evaluation of the protector activity against the T. cruzi infection will be made in mice treated with the glycopeptides or by the passive transference of its antibodies. The analysis of this protector effect will be made by the evaluation of the parasitaemia, determination of parasite load, heart histopathology analysis, leukocyte immunophenotyping, and cytokines quantification. It is understood that the development of this project may bring applicable data for the development of new terapeutic/diagnostic strategies for the Chagas disease.

Keywords: Glycopeptides; Trypanosoma cruzi; Mucins; Antibodies; Chagas disease; Diagnostic.

Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnólogico -CNPq

P15 - Comparative analysis of the genome, transcriptome and phenotypic of Salmonella Typhimurium strains isolated from humans and food in Brazil

Seribelli, A. A.[1] and Falcão, J. P.[1]

1 University of São Paulo - USP, School of Pharmaceutical Sciences of Ribeirão Preto, Department Clinical Analyses, Toxicology and Food Science, Brazil.

Salmonella enterica subsp. enterica serovar Typhimurium (S. Typhimurium) is one of the leading causes of gastroenteritis worldwide. Approximately 93.8 million cases of salmonellosis occur annually by non-typhoid Salmonella around the world. However, in Brazil there are few studies that have elucidated possible differences in the pathogenicity, virulence and genotypic diversity of S. Typhimurium strains isolated from humans and food. This project aims to compare the genome, to analyze the transcriptome and the phenotypic profile of S. Typhimurium strains isolated from humans and food during 31 years in different regions in Brazil. The comparative genome analysis will be performed for 92 strains isolated from humans (43) and food (49). For 40 strains isolated from humans (20) and food (20), it will be performed the phenotypic tests of epithelial cell invasion (CACO-2), macrophages survival test (J774), acid and oxidative stress tolerance tests. Moreover, the transcriptomes analysis of two S. Typhimurium strains will be performed, the RNAs will be extracted after the tests of tolerance to acid and oxidative stress and under ideal growth conditions for such strains. In addition, the median lethal dose (LD50) of two S. Typhimurium strains selected will be determined. It should be noted that there are no published studies comparing the S. Typhimurium strains isolated during different periods and sources in the country. There are no published data that gathered the analysis of the complete genomes, transcriptomes and phenotypic tests mentioned above. Therefore, the results to be obtained will probably contribute for a better characterization of the pathogenicity, virulence and genotypic diversity of this global important enteropathogen.

Keywords: Salmonella Typhimurium; complete genome; transcriptome; phenotypic tests.

Financial support: CAPES and FAPESP.

P16 - PHENOTYPIC COMPARISON AND PRESENCE OF VIRULENCE GENES AMONG CAMPYLOBACTER COLI STRAINS ISOLATED IN BRAZIL

GOMES, C.N.[1]; PASSAGLIA, J[1]; VILELA, F. P[1]; PEREIRA, F.M.H.S[1]; DUQUE, S. S[2]; J.P. FALCÃO[1]

1 SCHOOL OF PHARMACEUTICAL SCIENCE OF RIBEIRÃO PRETO (FCFRP-USP) UNIVERSITY OF SÃO PAULO (AV. DO CAFÉ S/N BAIRRO: MONTE ALEGRE, CEP: 14040-903, RIBEIRÃO PRETO - SP) 2 OSWALDO CRUZ FOUNDATION (FIOCRUZ), (AV: BRASIL, 4365 PAVILHÃO ROCHA LIMA, SALA 416, CEP: 21040-900, RIO DE JANEIRO - RJ).

Abstract: Campylobacter coli is an important causative agent of human diarrheal diseases worldwide. However, in Brazil it has not been frequently studied. The aims of this study were to analyze the effect of low temperatures in the growth of C. coli and to investigate the presence of some virulence genes of C. coli strains isolated in Brazil. A total of 50 C. coli strains isolated in Brazil from human feces (12), food (8) animals (15) and the environment (15) between 1995-2011 and two control strains, Salmonella Typhimurium ATCC 14028 and Campylobacter jejuni ATCC 3329, were studied. For the phenotypic test, the strains were grown at 42°C overnight on BBL Columbia Agar Base, supplemented with charcoal and FBP [0.05 % ferrous sulphate, 0.05 % sodium pyruvate and 0.05 %sodium metabisulphite diluted in sterile water] under microaerobic conditions. After incubation, a single colony from the plate was collected and inoculated in 9 mL of BHI broth and incubated at 42 °C under a microaerobic atmosphere for 16 h to obtain stationary phase cells that were used to analyze the effects of low temperature storage at 4 °C for 24 hours. The phenotypic experiments were conducted in three independent replicates and a statistical analysis was performed. The presence of 11 virulence genes was searched by PCR. All the strains of C. coli studied grew after 24 hours at 4 °C. There was no significant growth difference when comparing the studied strains with the control strains. All strains presented the flaA, cadF and sodB genes. The cdtB gene was detected in 13 (26%) strains; the flhA gene was detected in 11 (22%) strains; the dnaJ gene was detected in 9 (18%) strains; the pldA gene was detected in 7 (14%) strains ; the iamA gene was detected in three (6%) strains; the cdtC and docA genes were found in two (4%) strains; the cdtA and crsA were found in one (2%) strain and the ciaB, wlaN, virB11 and racR genes were not detected. The C. coli survival rates at low temperature indicate that better control measures may be needed given the importance of foods as vehicles of C. coli along with the extensive use of low temperatures for preservation. The presence of important virulence genes indicates the pathogenic potential of those strains.

Keywords: Campylobacter coli, virulence genes, effect of low temperature, Brazil

Development Agency: FAPESP

P17 - Molecular characterization and resistance profile of Shigella flexneri strains isolated during 34 years of different States of Brazil

GONZALES, J. C.[1], SERIBELLI, A. A.[1], PASSAGLIA, J.[1], FALCÃO, J. P.[1]

1 School of Pharmaceutical Sciences of Ribeirão Preto - University of São Paulo (FCFRP-USP)

According to OMS every year around 1.1 million deaths from shigellosis occurs worldwide. The main symptoms of this disease are diarrhea, dysentery and abdominal cramps. It is an important cause of morbidity and mortality, especially in underdeveloped countries. Monitoring of resistant strains of Shigella spp. is essential, since it guarantees that the efficient treatment. In addition, an increase in the antimicrobials resistance profile of Shigella spp has been reported. There are four species of Shigella: S. flexneri, S. sonnei, S. boydii and S. dysenteriae. In developing countries like Brazil, endemic shigellosis is caused by S. flexneri. The aims of this study are to analyze the pathogenic potential, genotypic diversity and antimicrobial resistance profile of strains of S. flexneri isolated for more than three decades in different States of Brazil. In this study 140 strains of S. flexneri isolated from human diarrheal feces from 1983 to 2017 will be studied. The presence of the 16 virulence genes will be verified by PCR. The susceptibility profile to 16 antimicrobials will be verified by the disc diffusion method. Based on these results, the presence of genes that confer resistance to β-lactam antibiotics will be investigated. The strains will be molecularly typed by the Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and Pulsed-field gel electrophoresis (PFGE) techniques. Thirty strains will be selected to be typed by Multi-locus Sequence Typing (MLST). Depending on the subtypes found by the mentioned techniques above, the complete genome sequencing of twenty representative strains of these subtypes will be done to obtain supplementary information about these strains. The results obtained should contribute to a better understanding of the genotypic diversity, pathogenic potential, profile and mechanisms of antimicrobial resistance strains of S. flexneri isolated for more than thirty years in several States of the country.

Keywords: Shigella flexneri, virulence genes, antimicrobial resistance profile, antimicrobial resistance genes, molecular techniques and complete genome sequencing.

Financial Support: CAPES e FAPESP

P18 - High concentration of 2,4-D herbicide increases the tfdA gene on soil.

Jéssica Aparecida Silva Moretto, Vânia Santos Braz, Lucas Miguel Altarugio and Eliana Guedes Stehling.

The increased use of pesticides to eradicate pests and weeds has resulted in changes in microbial biomass and formation of large amounts of toxic waste in soil. Thereby, the goal of this study was to evaluate if the selective pressure exerted by the presence of the 2,4-D herbicide can change the bacterial community structure of soil and quantify the number of copies of the tfdA gene, which is involved in the degradation of 2,4-D. In an agricultural experimental area, three mesocosms were constructed, each with an area of 9 m², in triplicate. A mesocosm was used as negative control, without application of herbicide and in the others were applied different concentrations of the herbicide. The sampling procedure followed a scale based on weeks (1st, 2nd, 3rd, 4th, 8th, 12th) and soil samples were collected at a depth of 5 to 10 cm from each mesocosm. Total DNA was extracted directly from soil samples following the time scale and used in the qPCR technique. The qPCR reactions were performed with samples treated with two different concentrations of 2,4-D (1x and 10x), in addition to the negative controls, over the 12 weeks. The results obtained have demostrated that the transient increase in the amount of tfdA in the soil probably reflects an increase in the size of the microbial community having the tfdA gene and / or increase in the copy number of the plasmids carrying it. Thus, the results present important information for a better understanding of the 2,4-D effects on the soil microbiota.

Keywords: pesticide; herbicide; 2,4-D; tfdA; soil; qPCR.

Financial Support: FAPESP and CAPES.

P19 - The role of Triiodothyronine (T3) in Chagas disease experimental

Pedro Alexandre S. Oliveira, Vânia Brazão, Luís M. Balbierato, Rafaela P. Colato, Lucas F. Tazinafo, José C. do Prado Jr.

American Trypanosomiasis, also known as Chagas disease, is a potentially life-threatening zoonotic illness caused by the parasite Trypanosoma cruzi. The disease is most commonly seen in Central and South America, Trinidad, and the southern United States. Often individuals susceptible to the parasite, that is, patients who develop clinical symptomatology to the infection, present several cardiovascular complications, with intense local inflammation, resulting in abnormal heart rhythm and compromised tissue functionality. In this context, the understanding of the cellular and immunological mechanisms that help in the control of the infection become of extreme importance. Of great relevance among immune response modulators, the iodinated hormones generated by the thyroid gland (T3 and T4) play a direct role in cardiomyocytes and all cardiac tissue, since the decrease or excess in hormonal production affects the activity from heart. However, the role of these hormones in chagasic infection remains poorly described. In this context, our group aims to understand the role of T3 during T cruzi experimental infection. For this, we will perform an exogenous treatment of T3 in infected animals and investigate several parameters, such as parasite load, cell migration, cytokine production, phagocytosis assays, killing capacity, cell death assay and dosage of cardiac injury markers. We believe that the elucidation of this mechanism may promote the development of new therapeutic interventions that confer protection to the host.

Keywords: Triiodothyronine, Chagas disease, immune response.

Financial Support: CAPES, CNPQ e FAPESP

P20 - Deregulation of Hippo pathway in Ph-negative myeloproliferative neoplasms patients

Cacemiro MC[1], Cominal JG[1], Souto EX[2], Simões BP[3], Castro FA[1]

1 DACTB Faculdade de Ciências Farmacêuticas de Ribeirão Preto - USP

2 Hospital Estadual de Transplantes Euryclides de Jesus Zerbini

3 Departamento de Clínica Médica Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto-USP

Background: The Hippo pathway, recognized as tumor suppressor pathway, regulates the cell number by modulating cell proliferation, cell death, and cell differentiation. This pathway is composed by MST1/2, SAV1, Lats1/2, MOB1 and YAP/TAZ and the deregulation of this pathway has been related to the development of several cancer types. In this way, considering the important role of this signaling pathway in cell proliferation and death and the participation in many cancers development, we decided to investigate the relationship between the Hippo pathway and the pathogenesis of myeloproliferative neoplasms (MPN), that are clonal disorders resulting from the transformation of hematopoietic stem cell (HSC), leading to an increase in mature blood cells, comprising disorders like essential thrombocythemia (ET) polycythemia vera (PV) and primary myelofibrosis (PMF). The JAKV617F is the major genetic event driving the phenotype of these disorders, however, another mutation recently discovered called CALR has been related to the MPN pathogenesis. Aims: To quantify the MST1, MOB1B/A, YAP, TAZ, LATS1 and LATS2 gene expression in MPN patients and controls subjects (CTRL) and to evaluate the influence of JAK2V617F and CALR mutation in the Hippo pathway gene expression. Subjects and Methods: The peripheral blood leukocytes were obtained from 36 PV patients (median age = 61 years; 16 men and 20 women), 36 ET patients (median age = 59 years; 10 men and 26 women), 24 PMF patients (median age = 64,5 years; 17 men and 7 women) and 60 CTRL subjects (median age = 55 years; 21 men and 39 women). The RNA was extracted by Trizol® method and the cDNA were synthesized using High Capacity cDNA reverse transcription kit®. The gene expression was assessed by real time PCR by using the TaqMan gene expression assays® and the results were expressed as relative units of expression. Results: In PV patients, we observed decreased levels of MST1 (median=71.17; $p=0.0072$), MST2 (median=98.14; $p<0.0001$) and LATS2 (median=501.5; $p=0.0295$) in comparison to CTRL (median=139.0, median=664.3 and median=867.7, respectively; $p<0.05$). Moreover, the JAK2V617F mutation did not affect the Hippo pathway gene expression in these patients. Concerning to ET patients, we observed decreased levels of MST1 (median=83.47; $p=0.0147$), LATS1 (median=172.8; $p=0.0295$), LATS2 (median=433.8; $p=0.0090$) and SAV1 (median=89.77; $p<0.0001$), in comparison to CTRL (median=139.0; median=298.6; median=867.7 and median=736.8 respectively; $p<0.05$). In the same way, the JAK2V617F mutation did not affect the Hippo pathway gene expression, however, the CALR positive patients presented increased levels

of LATS1 (median=291,37; p=0.0206), MOB1B (median=2209,6; p=0.0037), and SAV1 (median=153,32; p=0.0037) in comparison to the CALR negative patients (median=76.76; median=670.33; median=39.08; respectively; p>0.05). In the PMF patients we observed decreased levels of SAV1 (median=66.16; p<0.0001), and TAZ (median=107.4; p=0.0011) in comparison to CTRL (median=298.6; median=229.1; respectively). In these patients, we also observed increased expression of MST1 (median=112.26; p=0.0421) in the JAK2V617F-mutated patients in comparison to non-mutated patients (median=222.97), but the CALR mutation did not affect the Hippo pathway gene expression in these patients. Conclusion: The results indicate that MPN patients present deregulated Hippo pathway gene expression which can contribute to the MPN pathogenesis, since this signaling pathway is important in the control of cell proliferation and death process.

Keywords: Hippo Pathway, myeloproliferative neoplasms, polycythemia vera, essential thrombocythemia, primary myelofibrosis.

Supported by FAPESP (2014/04234-9).

P21 - Evaluation of photodynamic inactivation of Fusarium solani and Candida albicans using nanoencapsuled phenothiazinum photosensitizers

Glaucia Rigotto Caruso, Henrique Dantas de Menezes, Priscyla Daniely Marcato Gaspari, Luciano Bachmann, Mark Wainwright, Gilberto Úbida Leite Braga, Marcia Regina Von Zeska Kress.

Aims: The emergence of pathogenic fungi and resistance to commercial antifungal drugs leads to the urgent need to develop new strategies for treating fungal infections. The antimicrobial photodynamic therapy (APDT) is a promising technique to control resistant microorganism species due to the multi target activity. Additionally, the correct delivery of phenothiazinium photosensitizers (PS) in the infected host can increase the efficiency of the antifungal process. In this scenario, this work aims to: (1) Synthesize and characterize liposomes using different compositions (phosphatidylcholine, cholesterol and ergosterol) with PS; (2) Evaluate the fungicidal and fungistatic activity of encapsulated PS in comparison to PS alone; (3) Determinate optimal parameters (concentration of liposomes containing PS, incubation time of the cell with liposomes, and time of exposure to light) for APDT of Candida albicans and Fusarium solani yeast cell and microconidia, respectively; (4) Study of liposomes internalization and membrane interactions with the fungal cells (yeast and microconidia); (5) Evaluate the lipid and protein damage to the fungal cell caused by APDT.

Methods: The liposomes will be synthesized using the ethanolic injection method proposed by Batzri e Korn (1975). The characterization will be performed using dynamic light scattering method to obtain the diameter and charge of the liposomes. Transmisson eletronic microscopy will be used to evaluate liposomes morphology The minimal inhibitory concentration (MIC) and survival fraction method will be used to evaluate the optimal conditions for APDT and to determine fungicidal and fungistatic effect, respectively; To evaluate and quantify the PS internalization and subcellular localization, confocal microscopy will be used; The lipid peroxidation and protein carbonilation of the microconidia and yeast cell after APDT will be evaluated by “Lipid Peroxidation Assay Kit” and “Protein Carbonyl Assay Kit” (Sigma-Aldrich, Inc., MO, EUA), respectively , both following manufacturer's instructions. Statistical analysis will be performed using one-way ANOVA and Tukey’s test to compare the effect of different treatments.

Keywords: antimicrobial photodynamic therapy; liposomes; phenothiazinium; F. solani; C. albicans

Financial Support: Coordenação de Aperfeiçoamento de Pessoa de Nível Superior (CAPES); Fundação de Amparo do Estado de São Paulo (FAPESP).

P22 - Development of real-time RT-PCR for differential diagnosis of circulating arboviruses in Brazil

Acchite, AL, Lippi, Veronica, Trabuco, AC, Aquino, VH.

FCFRP-USP, Ribeirão Preto, Brazil

Arthropod-born viruses are classified informally as arboviruses. More than 150 arboviruses infect humans causing febrile illness, as well as hemorrhagic and central nervous system diseases that can be fatal in some cases. Arboviroses are of great importance for public health in tropical countries like Brazil. The most widespread arboviruses in Brazil are: Dengue virus (DENV), Chikungunya virus (CHIKV), Zika virus (ZIKV), Oropouche virus (OROV) and Yellow Fever virus (YFV). Other flaviviruses with clinical relevance have already been isolated in Brazil, such as Saint Louis encephalitis virus (SLEV), Rocio virus (ROCV), Cacipacore virus (CPCV), Ilheus virus (ILHV), Bussuquara virus (BUSV), Iguape virus (IGUV) and Mayaro virus. The development of highly sensitive and specific methods for detecting the infection caused by these viruses is of great importance for early diagnosis, allowing better clinical and preventive measures to reduce the risk of epidemics. The aim of this work is to develop real-time RT-PCR methods for detection of CPCV, ILHV, BUSV, IGUV, MAYV, YFV, SLEV, OROV and WNV. Specific primers and probes for each of these viruses will be designed by selecting conserved regions by aligning viral genomic sequences deposited in GenBank, using the CLC Main Workbench software. The primers will be designed for amplification of genomic regions of 120-200 base pairs, and the probes in regions internal to these regions. Temperature of annealing and concentration of primers and probes will be analyzed to obtain the best amplification conditions and consequent viral detection. Finally, the sensitivity and specificity of the RT-PCRs will be analyzed.

Keywords: Arboviruses, Real Time RT-PCR, Diagnosis.

P23 - Initial in silico characterization of Neospora caninum antioxidant enzymes

Jade Cabestre, Venancio Brochia, Luiz Miguel Pereiraa, Luciana Baronia, Ana Patrícia Yatsuda Natsuia

a. School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo. Department of Clinical Analysis, Toxicology and Food Science.

Neospora caninum is an Apicomplexa protozoan associated with neurological diseases in dogs and abortions in cattle. This parasite depends on an enzymatic antioxidant system defense for evading the oxidative stress inside the host cell and, ensuring a successful intracellular replication. Among these enzymes, peroxiredoxin 1 (NcPrx1) and glutathione reductase (NcGR) draw our attention because both are present in significant relative abundance in Neospora caninum and no studies have been performed. Therefore, our aim was an initial in silico analysis of the N. caninum antioxidant enzymes peroxiredoxin 1 and glutathione reductase. The sequences of NcPrx (NcLIV_062630) and NcGR (NcLIV_063590) were submitted to the BLASTp 2.0 and were checked for the presence of signal peptide (SignalP 4.1) and domains (Pfam 3). The predicted aminoacid sequences of the NcPrx and NcGR were aligned with homologous apicomplexan enzymes (MegAlign software followed by GeneDoc). NcPrx revealed homology with the peroxiredoxin genes from Besnoitia besnoiti; Plasmodium berghei ANKA and Toxoplasma gondii, with identity/similarity values (I/S%) of 87/ 93%; 88/94% and 88/94%, respectively. Specific domains were found in NcPrx, indicating that this protein belongs to the alkyl hydroperoxide reductase (AhpC) and thiol specific antioxidant (TSA) superfamily and thioredoxin (TRX)-like superfamily. NcPrx was described as a mitochondrial enzyme with its active site on amino acid 51 (UniProt). NcGR revealed homology with the GR proteins from T. gondii M49 (I/S:86/92%), H. hammondi (I/S:86/93%), B. besnoiti (I/S:73/82%). NcGR belongs to the pyridine superfamilies nucleotide-disulphide oxidoreductase and NAD(P)H-nitrite reductase(Nirb) superfamily. As expected, no signal peptide was detected on both NcPrx and NcGR. Thus, the results demonstrated high conservation of the enzymes peroxiredoxin 1 and glutathione reductase from N. caninum compared to representatives of Apicomplexas. The project is continuing with the cloning of NcPrx1 and NcGR genes, expression and purification of the recombinant enzymes for further molecular characterization in N. caninum.

Keywords (up to six words separated by semicolons): Neospora caninum; Apicomplexa; antioxidant; Peroxiredoxin; Glutathione reductase

Financial Support. Capes

P24 - Metagenomic Prospection of Quorum Sensing Related Bacteria During Spontaneous Cocoa Beans Fermentation

Almeida, O.G.G.[1]; Martinis, E.C.P[1].

[1] Departament of Clinical Analysis, Toxicology and Food Sciences, FCFRP-USP.

The process of fermentation of cocoa beans is not well standardized, since the it is carried out by microorganisms that are autochthonous from cocoa beans. It is a highly complex process because there is a microbial succession that starts with yeasts and it is mantained and finished by lactic acid and acetic acid bacteria. In this way, many authors tried to achieve a bacterial starter culture to guarantee the standardization in the characteristic chocolate flavor, that is dependent of microbial metabolites released during this process. Nevertheless, it is not described yet in literature a starter culture that may be applied successfully in the fermentative process. Moreover, some authors mentioned the quality of chocolate might be related to the presence of genes involved in Quorum Sensing (QS) or Quorum Quenching (QQ) classic pathways. In that context, it is important to understand the dynamic of the fermentation process from the bacterial communication point of the view, isolating strains that dominate in process to develop, after phenotypic characterization, a starter well defined culture. In conclusion, our goals are: (I) study the microbiota of cocoa beans fermentation during specific times; (II) To sequence genomic DNA extracted from fermentation samples and to analyze with Illumina HiSeq 2500 platform; (III) to do bioinformatic analysis to identify bacterial strains that express genes related to QS and QQ; (IV) Enumerate, isolate and perform phenotypic analysis in QS bioassays to measure QS expression genes in vitro; (V) Use HPLC-MS/MS to quantify autoinducers molecules produced by isolated bacterial cultures; (VI) Propose a starter culture with well-defined bacterial populations for cocoa fermentation. The results of this project should contribute for improvement and selection of flavors during cocoa beans fermentation. The authors thanks to FAPESP (process 2017/13759-6) by the financial support in this research.

Key-words: cocoa fermentation, quorum sensing, metagenomics.

P25 - GH: Its administration and the pathways involved in immune response and cardioprotection during experimental Chagas' disease

Luis Marcio Balbierato[1], José C. do Prado Jr.[1]

1 Departamento de Análises Clínicas Toxicológia e Bromatologia. Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Brazil.

Chagas disease is one of the most important neglected diseases in Latin America, reaching approximately 8 million infected in the chronic phase worldwide. Although the immunological mechanisms that confer protection or promote the formation of cardiac lesions during Trypanosoma cruzi infection have not yet been fully elucidated, studies show that chronic Chagas' heart disease presents a worse prognosis than dilated cardiomyopathies of a noninflammatory nature, as a consequence of the response exacerbated immune system. Among the many pathologies caused, the cardiac system seems to be of greater importance, presenting mild ventricular hypertrophy, tachycardia, and repolarization disorders during the acute phase, such as cardiomegaly and cardiac insufficiency in the chronic phase. Some studies suggest an important role for the GH / IGF-I axis in the regulation of cardiac function. In addition, growth hormone-releasing hypothalamic hormones (GH), such as GHRH, exert beneficial effects on the cardiac system. Given this and studies demonstrating the reduction of GH levels in patients with congestive cardiomyopathy, this project aims to evaluate the possible cardioprotective effect subsequent to the administration of GH during the chronic phase of the infection. For this, different parameters will be evaluated: Immunological (Dosing of cytokines TNF-α, IFN-γ, IL2, TGF-Beta by ELISA and Cellular population by flow cytometry, plasma levels of the following hormones T4, T3, TSH, GH, prolactin, free testosterone, IGF-1 and corticosterone, cardiac injury markers (Troponin, Ck-MB and C-reactive protein) and histopathology of the heart.

Key words: Trypanosoma cruzi, Growth hormone (GH), Chagas' disease

Financial Support – CAPES

P26 - Characterization and the increase in production scale of the recombinant human granulocyte colony stimulating factor (rhG-CSF) produced in Pichia pastoris

Bruna Samhan Archangelo; Elisa Maria de Sousa Russo

Human granulocyte colony stimulating factor (hG-CSF) acts primarily to promote the maturation of neutrophils (granulocytes) and stimulate their phagocytic and chemotactic activity. It has been used in the treatment of various pathologies, especially neutropenia caused by events that suppress the production of myeloid cells. The use of this biopharmaceutical has been released by the US regulatory agency (FDA) since 1991. In Brazil, this drug is part of the SUS Pharmaceutical Assistence Program as an exceptional medicine, but it has a very high cost for procurement. The Pichia pastoris yeast has interesting characteristics as an expression system with the possibility of simplified genetic modification, the ability to secrete proteins and to perform post-translational modifications, besides being a organism of easy manipulation. Our group has already performed the cloning and the expression of rhG-CSF in P. pastoris. However, the production occurred only on a smaller scale. This project aims to increase the scale of production of the human recombinant protein G-CSF in bioreactor, perform in vitro and in vivo biological tests and perform an analysis of the glycosilation patterns of the recombinant protein produced.

P27 - STUDY OF OXIDATIVE METABOLISM OF NEUTROPHILS EXPOSED TO SEAWEED OR ENDOPHYTIC FUNGI COMPOUNDS FROM ANTARCTIC

NOGUEIRA, G. A[1]; MORAES, V. M.[2]; TUCCI, L. F. F.[1]; DEBONSI, H. M.[2]; CANICOBA, N. C.[1];; MARZOCCHI-MACHADO, C. M.[1]

[1]: School of Pharmaceutical Sciences of Ribeirão Preto - USP, Department of Clinical Analyses, Toxicology and Food Science

[2]: School of Pharmaceutical Sciences of Ribeirão Preto - USP, Department of Chemistry and Biological Physics

Aim: To evaluate the action of natural compounds in the regulation of neutrophil oxidative metabolism.

Methods: The crude extract of Palmaria decipiens and nine (9) fractions of the endophytic fungi Aspergillus unguis have been studied in the present research. The scavenger activity was evaluated by α, α-diphenyl-β-picrylhydrazyl (DPPH), as a cell free assay; Cell viability was evaluated by Trypan Blue and release of Lactate-desydrogenase (LDH) enzyme; Reactive Oxygen Species (ROS) production was analyzed by luminol-dependent chemiluminescence (QL-lum) with phorbol 12-myristate 13-acetate (PMA) and Zymosan opsonized with normal human serum (NHS) as independent and dependent receptor activation stimuli, respectively.

Results: Aspergillus unguis fractions FR5 and FR6*** presented intermediary QL-lum interference and viability (51% and 92±0; 95% and 74±10, respectively). No products presented scavenger activity.

Conclusion: Two of ten natural products showed an intermediary effect reducing the measurement of the ROS. Further investigation is required about chemical properties' compounds and optimization for application in vitro analyses.

Keywords: Neutrophil; Oxidative Metabolism; Natural compounds.

Financial Support: Coordination for the Improvement of Higher Education Personnel (CAPES)

P28 - Evaluation of naive T and B cell generation in patients with sickle cell anemia treated with different therapeutic modalities

Luciana Ribeiro Jarduli[1], Kelen Cristina Ribeiro Malmegrim de Farias[1]

1 Departamento de Análises Clínicas Toxicológia e Bromatologia. Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Brazil.

Aim: To evaluate the T and B-cell ontogenesis in sickle cell anemia (SCA) patients treated with hydroxyurea (HU), chronic transfusions or allogeneic hematopoietic stem cell transplantation (HSCT). SCA patients have deregulated immune function and frequent infection episodes. Methods: Thymic function was evaluated by RT-qPCR quantification of β- and signal joint (sj)-T-cell receptor excision circles (sjTREC) and intra-thymic T-cell division (n) was calculated by the formula: n=Log(sjTREC/βTREC)/Log2. B cell generation was measured by coding-joint (Cj) and sj-kappa-deleting recombination excision circles (sjKREC), and B-cell divisions in the peripheral blood were calculated by the formula: n=Log(Cj/sjKREC)/Log2. Results: Patients with SCA without any treatment showed decreased sjTREC and β-TREC levels. Thymic function recovered at one year after HSCT, and patients who developed acute graft-versus-host disease showed a significant decreased of sjTREC and β-TREC levels at 6 months post-transplantation. Disturbed B-cell homeostasis was found in patients with SCA with important increase of sjKRECs and Cj in patients treated with HU or chronic transfusions. In addition, a significative increase of sjKRECs and Cj was found after transplantation, reflecting the effective naive B-cell reconstitution in SCA patients following HSCT. Conclusions. Despite of the inflammatory environment, a preserved naive T and B cell regeneration by was found in the SCA patients following HSCT. Treatment of SCA patients with HU or chronic transfusions also improves the thymus and bone marrow function, although to a lesser extent. All together, our results demonstrated the positive effect of these therapies, especially the HSCT, on the immune status of SCA patients.

Keywords: Sickle cell anemia; Thymic function; Hydroxyurea; Chronic transfusions; Allogeneic Hematopoietic Stem cell Transplantation.

Financial Support: FAPESP (grant#: 2014/03866-1; grant#: 2016/11544-0)

P29 - EFFECTS OF EXTRACTS AND FRACTIONS OF MARINE ALGAE AND ENDOPHYTIC MICROORGANISMS ON NEUTROPHILS FUNCTIONS MEDIATED BY Fcγ AND COMPLEMENT RECEPTORS

NATHÁLIA CRISTINA CANICOBA[1]; HOSANA MARIA DEBONSI[2]; CLENI MARA MARZOCCHI MACHADO[3].

1,2,3 Faculdade de Ciências Farmacêuticas de Ribeirão Preto – FCFRP/USP

Introduction: Systemic lupus erythematosus (SLE), an autoimmune disease has a pathophysiology, which is known by the neutrophils involvement. Several immunoreceptors are involved in the activation or inhibition of neutrophils. Among these receptors, the IgG (Fc□) and complement receptors had changes described in SLE. Currently SLE treatment is based on immunosuppressive therapies, which are associated with toxicity. The challenge of develop a new therapy is how to create a therapeutic target that controls the deleterious responses of neutrophils without impairing their important functions for the maintenance of homeostasis. Therefore, our research group has studied the microorganism associated with marine algae due to their ecological relations and/or biochemical interactions. Aim: To evaluate the effect of extracts of the marine algae Gigartina sp and fractions of endophytic microorganisms isolated from this algae on the neutrophils functions mediated by Fcγ receptors and complement receptors. Methods: Healthy and lupus neutrophils will be stimulated or not with zimosan, immune complexes – both opsonized with normal human serum (NHS) and treated with inactive human serum (IHS) – and phorbol 12-myristate 13-acetate (PMA); oxidative burst will be evaluated by luminol-dependent chemiluminescence; human umbilical vein endothelial cell (HUVEC) will be exposed to healthy and lupus neutrophils; HUVEC aggression will be evaluated by the measure of lipid peroxidation by the thiobarbituric acid reactive substance methods; myeloperoxidase transfer from neutrophils to HUVEC will be analyzed by flow cytometry with specific monoclonal antibody and the endothelial activation will be assessed by the release of ICAM-1 by enzymatic assay. Results: the extract and the fractions were efficient to decrease neutrophils oxidative burst of healthy donors. Conclusion: The data presented here suggest that these extracts and fractions may act as antioxidants in neutrophils. It is expected that this work contribute to the search for new bioactive molecules to regulate neutrophil-mediated responses.

Keywords: neutrophils; systemic lupus erythematosus; Fcγ receptor (SLE); complement receptor; algae; microorganism.

Financial Support: CAPES

P30 - Actions of Sphingosine-1-phosphate analogue (FTY720) in head and neck squamous cell carcinoma

SANTOS, L.F.[1]; SANTOS, T.P.M.[1]; FUGIO, L.B.[1]; BRAUER, V.S.[1]; NETO, M.P.C.[1]; GARCIA, C.B.[1]; SOUSA, L.O.[1]; CURTI, C.[2]; LEOPOLDINO, A.M.[1]

1 Department of Clinical Analyses, Toxicology and Food Sciences, and 2 Dept of Physics and Chemistry, School of Pharmaceutical Sciences of Ribeirão Preto, University of Sao Paulo, Ribeirão Preto, Brazil.

The main effects of FTY720, a sphingosine analogue, are as antagonist of sphingosine kinase 1 (SphK1) and activator of phosphatase 2A (PP2A). Due to its action as an immunosuppressant, this pro-drug has been used for the treatment of multiple sclerosis patients. FTY720 has been proposed as an antitumoral strategy in different cancer types, such as prostate, breast, several forms of leukemia and lymphoma, lung, liver, pancreatic, bladder, renal, glioma, gastric, colon and ovarian. However, FTY720 action in head and neck squamous cell carcinoma (HNSCC) has not yet been investigated. Objective: the present study addressed the potential antitumoral of FTY720 in HNSCC. Methods: PP2A activity was assessed in HNSCC. Cells were treated with FTY720 and several target proteins of S1P signaling pathways were analyzed by Western blot. Flow Cytometry was used to determine cell viability, and in the functional studies of cell death signaling using inhibitors for caspases (Q-VD-OPh), necroptosis (Necrostatin-1) and autophagy (3-Methyladenine). Proteasome inhibitor (MG132) was used to evaluate the response of cells under blocking of these processes. Results: A panel of HNSCC cell line was analyzed for PP2A activity, and SCC9 cell was selected for the mechanism studies. As expected, FTY720 increased phosphatases activity, including PP2A, decreased c-Myc and SphK1 protein levels, and induced activation of caspase-8 and -3 in SCC9 cells. Apoptosis signaling was blocked in SCC9 cells treated with pan-caspase inhibitor, and, consequently, cell viability was recovered. Necroptosis inhibition did not change cell viability, and autophagy inhibition drastically decreased it, suggesting that autophagy is a protective response in SCC9 cells. FTY720 showed promising effect regarding apoptosis induction in HNSCC. Compounds with potential to act synergistically with FTY720 are under investigation in our Laboratory, looking for a new strategy in HNSCC therapy.

Keywords: Sphingosine; head and neck cancer; SphK1; antitumoral therapy.

Financial Support. FAPESP, CEPID, CAPES and CNPq.

P31 - New rhoptry proteins (ROP) of the apicomplexan Neospora caninum: NcROP15B and NcROP55

Julia Audrey de Paula; Luiz Miguel Pereira; Luciana Baroni; Marcos Alexandre Bezerra; Maraisa Palhão Verri; Ana Patrícia Yatsuda Natsui

School of Pharmaceutical Sciences of Ribeirao Preto – University of São Paulo

Neospora caninum is a coccidian of the Apicomplexa phylum, described as the main cause of parasitic abortion in cattle (neosporosis), due to the high transmission efficiency of the infected cow to its fetus. Apicomplexans interact and invade the host cells through the coordinated protein secretion of the apical complex, composed of organelles called micronemes, rhoptries and dense granules. Rhoptries proteins are associated with the parasitophorous vacuole formation (PV), intracellular environment survival and parasite virulence. In N. caninum, only a few rhoptries proteins have been so far identified and characterized, such as ROP40 and NcROP2Fam-1. Thus the aim was to identify and characterize the NcROP15B (NcLiv_011700) and NcROP55 (NcLiv_031550) rhoptries proteins of N. caninum. In silico analyzes of the NcROP15B and NcROP55 sequences indicated the presence of a domain belonging to the protein kinase family for NcROP15B and NcROP55 and a signal peptide only for NcROP15B. The regions for recombinant expression were amplified by PCR from the N. caninum tachyzoite cDNA. The inserts were subcloned (pGEM) and then ligated into pET28 expression plasmid in E. coli BL21 (DE3) for IPTG-induced recombinant expression. After purification, the recombinant NcROP15B and NcROP55 were used for immunization of mice. Both anti-ROP15B and anti-ROP55 polyclonal serum reacted with two proteins in the N. caninum extract in Western Blot 1D, with approximately 35 KDa, within the predicted for NcROP15B (32 KDa), but below for NcROP55 (47.9 KDa). In confocal immunofluorescence, NcROP-15B and NcROP55 exhibited a perinuclear localization pattern. Further investigation of NcROP-15B and NcROP55 are being performed for a better understanding of these protein kinases associated with the processes of invasion and proliferation of the parasite, targets with great preventive potential.

Keywords: rhoptry;Neospora caninum;Apicomplexa

Financial Support: CAPES and CNPq

P32 - Morphological and functional characteristics of multipotent mesenchymal stromal cells of Philadelphia-negative myeloproliferative neoplasms

Cominal JG[1], Cacemiro MC[1], Bertolino GM[1], Ambrosio L[1], Mizukami A[2], Kashima S[2], Figueiredo-Pontes LL[2,3], Simões BP[2,3], Covas DT[2,3], Malmegrim KCR[1,2], Castro FA[1,2].

1 Department of Clinical Analyses, Toxicology and Food Science, University of São Paulo at School of Pharmaceutical Sciences of Ribeirão Preto, Ribeirão Preto, Brazil.

2 Hemotherapy Center of Ribeirão Preto, University of São Paulo at Ribeirão Preto Medical School, Ribeirão Preto, Brazil.

3 Department of Internal Medicine, University of São Paulo at Ribeirão Preto Medical School, Ribeirão Preto, Brazil.

4 Klinikum Schwabing, Müenchen, Germany.

Multipotent mesenchymal stromal cells (MSC) are essential components of hematopoeitic niches, support and regulate the fate of hematopoietic stem cells (HSC) and the hematopoiesis process. Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) are clonal disorders of HSC characterized by accumulation of mature myeloid cells and classified as Philadelphia-negative myeloproliferative neoplasms (MPN). MPN's pathogenesis has been related to driving genetic events and alterations in the hematopoietic niches. Our hypothesis is that MSC from MPN patients have intrinsic alterations possibly involved in the pathogenesis of these diseases. Aims: To isolate MSC from bone marrow (BM-MSC) of patients with MPN and characterize their proliferative capacity, differentiation potential and immunophenotic profiles. Methods: BM-MSC were isolated from 5 PV, 3 ET and 3 PMF patients. BM-MSC were cultured in α-MEM medium supplemented with 1% de penicillin/streptomycin, and 15% FBS, at 37ºC with 5% CO2 for five passages. Differentiation potential in adipocytes and osteocytes was performed using specific differentiation media. Immunophenotyping was performed by flow cytometry. The doubling time (DT) of the cell population was calculated by a pre-established equation. Determination of maximum specific growth rates and the statistical analyses were performed via GraphPad Prism. Results: BM-MSC from PV, TE and PMF showed high expression of characteristic surface markers (CD90, CD73, CD105, CD166, HLA-ABC, and CD29) and differentiate into adipocytes and osteoblasts. The medians DT (between cell passages 1-3) were 115, 129 and 144 hours, of BM-MSC from PV, TE e PMF patients, respectively. Median fold-increase (FI) was 4.32 (PV), 2.85 (TE) and 2.92 (PMF) times. There were no statistically significant differences between analyzed parameters among the BM-MSC from PV, ET and PMF patients. Comparisons with BM-MSC isolated from healthy donors have yet to be performed. Conclusions: BM-MSC from MPN's patients showed characteristic in vitro proliferative capacity, immunophenotype and multipotent differentiation. Comparisons with healthy donors BM-MSC and epigenetic evaluations have yet to be performed to complete those results.

Keywords Multipotent mesenchymal stromal cells, myeloproliferative neoplasms, hematopoietic niche.

Financial Support CAPES, Center for Cell-based Therapy (CTC-CEPID-FAPESP) - Hemotherapy Center of Ribeirão Preto

P33 - Genotypic and phenotypic profile of Fusarium spp. isolated from clinical episodes of fusariosis in Sao Paulo State

Paziani, M. H.[1]; von Zeska Kress, M. R. [1]

1 School of Pharmaceutical Sciences of Ribeirão Preto – University of Sao Paulo

The human pathogenic filamentous fungus Fusarium spp. is cosmopolitan and prevalent in tropical regions. The asexual reproduction is characterized by micro and macroconidia production which are dispersed in the nature by wind, water or direct contact with the host. The occurrence of fusariosis is high in immunocompromised patients. Azoles and polyenes are the antifungal classes used in the treatment of fusariosis. However, the frequent resistance to these antifungals leads to the host death.F. oxysporum, F. solani, F. moliniforme, F. proliferatum and F. dimerum are the species described with a greater risk to human health. Biofilm is a virulence factor present in Fusarium species which are present in ophthalmic lenses and hospital water pipes, promoting ceratites and other systemic mycoses, respectively. Herein is proposed the phenotypic and genetic characterization of Fusarium spp. clinical isolates from different anatomical sites from Sao Paulo State patients. In this study was performed the identification of clinical isolates by classical and molecular methods. The susceptibility to commercial antifungal and antimicrobial photodynamic therapy will be evaluated. Additionally, in order to evaluate the fungal pathogenicity of the clinical isolates, we will evaluate both the biofilm capacity formation (biomass and matrix) by crystal violet and safranin methods, and the virulence with the alternative model Galleria mellonella. The prevalence of fusarioses is related to onychomycosis (66%), blood infections (19%), dermatomycoses (13%) and peritoneal fluid micoses (2%). A total of 108 isolates were identified, 93 belonging to F. solani complex (86%), 13 to F. oxysporum (12%), 1 to F. dimerum and 1 to F. fujikuroi (1%).

Keywords (Fusarium sp; fusariosis; phylogeny; biofilm; virulence).

Financial Support. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

P34 - HIPPO pathway's regulatory microRNAs in Chronic Myeloid Leukemia

Maria Gabriela Berzoti-Coelho[1], Sandra Mara Burin[1], Ana Paula Zambuzi Cardoso Marsola[1], Fabíola Attié de Castro[1].

1 School of Pharmaceutical Sciences of Ribeirão Preto - University of São Paulo (USP) - Ribeirão Preto – SP.

Aims: To investigate the HIPPO pathway's regulation by microRNAs (miRs) in Chronic Myeloid Leukemia (CML) and their role in CML pathogenesis and resistance to the tyrosine-kinase inhibitors (TKI) treatment. Since YAP and TAZ are the major downstream effectors of HIPPO pathway and well-known targets of miR-132 and miR-141, respectively, were evaluated the expression of these miRs in CML patients and cell lines. Subjects and Methods: Peripheral Blood Mononuclear Cells were obtained from 46 CML patients (median age = 48 years; 10 at CML diagnosis and 36 treated with TKI) and 20 controls (median age = 44 years). The KCL-22 S and KCL-22 R (sensitive and resistant to imatinib mesylate, respectively) cell lines were cultured in RPMI 1640 medium under 5% CO2 at 37°C. The miRs expression was assessed by RT-qPCR using TaqMan gene expression assays® and results were expressed as relative units of expression (RUE) or fold-change. Results: the miR-141 expression was lower in patients at CML diagnosis (RUE=1.14) when in comparison with TKI-treated patients (RUE=18.37; $p<0.0001$) and controls (RUE=20.47; $p<0.0001$). The controls (RUE=20.47) expressed more miR-141 than the TKI-treated patients (RUE=18.37; $p=0.05$). The miR-132 expression were higher in controls (RUE=390.3) than in patients at CML diagnosis (RUE=237.2; $p=0.0057$) and patients treated with TKI (RUE=219.6; $p<0.0001$). No difference in miR-132 expression was observed between CML patients at diagnosis or treated with TKI. The KCL-22 R cells (fold-change=0.38) expressed less miR-141 than KCL-22 S cells (fold-change=1.0; $p<0.05$). Regarding the miR-132 expression, no statistical difference was observed between KCL-22 R and KCL-22 S cells. Conclusion: The miR-141 and miR-132 downregulation in CML patients at diagnosis may result in the HIPPO pathway's deregulation, favoring the proliferation and oncogenic functions of YAP and TAZ. The lower expression of miR-141 may favor the proliferation and apoptosis resistance by TAZ up-regulation in KCL-22 R cells.

Keywords: miR-141; miR-132; HIPPO pathway; Chronic Myeloid Leukemia.

Financial Support: CAPES and FAPESP (process number 2015/23555-3).

P35 - PLASMID-MEDIATED QUINOLONE RESISTANCE (PMQR) AND EXTENDED-SPECTRUM BETA-LACTAMASES (ESBL) GENES IN CHROMOSOMAL AMPC-PRODUCING ENTEROBACTERIACEAE ISOLATED FROM HOSPITALIZED PATIENTS: QNRD1 PREVALENCE

ARAUJO, I.J.[1]; ANDRADE, L.N.[1]; FERREIRA, J.C[1]; MARTINEZ, R.[2]; DARINI, A.L.C.[1]

1 School of Pharmaceutical Sciences of Ribeirão Preto – University of São Paulo; 2 Clinical Hospital of Ribeirão Preto – University of São Paulo

Chromosomal AmpC-producing Enterobacteriaceae, especially the genera Serratia, Providencia, Citrobacter, Proteus and Morganella are opportunistic bacteria associated with nosocomial infections. It is verified that there are few phenotypic and molecular data about antimicrobial resistance for these species, when compared to others. This study aimed the investigation of plasmid mediated quinolone resistance (PMQR) and extended-spectrum beta-lactamases (ESBL) genes in chromosomal AmpC-producing Enterobacteriaceae resistant to quinolone and/or 3 rd -4 th generation cephalosporins. Bacteria were isolated from patients in a university hospital in two different time periods (2007, n=19 and 2016, n=48). Antimicrobial susceptibility test and double disc screening test (DDST) were realized aiming the obtention of the antimicrobial susceptibility profile, phenotipical detection of ESBL and overproduction of AmpC was carried out by combination disk test using cloxacillin We investigated the presence of PMQR genes (qnrA, B, C, D, S e VC, aac(6´)-Ib-cr, qepA and oqxAB) and ESBL genes (blaCTX-M, TEM and SHV) by PCR and sequencing. In addition, plasmid replicons of the major incompatibility (Inc) groups occurring in Enterobacteriaceae were screened by PCR-based replicon typing (PBRT) scheme, colE-like plasmids were also investigated. PFGE was realized in order to verify bacterial clonality. In 19 enterobacteria (Morganella morganii, n=9, Serratia marcescens, n=5, Citrobacter koseri, n=2, Citrobacter freundii, n=1, Providencia stuart n=1, Proteus mirabilis, n=1) qnrD1 gene was detected. Some M. morganii presented concomitantly other qnr genes (B or S). Moreover, blaCTX-M-2 was also detected in M. morganii. For other isolates, AmpC-overproducing was responsible to resistance to extended cephalosporins. colE-like plasmids were detected in almost all isolates. These pathogens seem to be important reservoir of qnr genes in the hospital studied, highlighting qnrD1, little reported so far compared to other qnr genes. It was also possible to observe increase frequency of qnr genes in these species comparing 2007 to 2016.

Keywords: qnr, ampC, CTX-M, plasmids, opportunistic pathogens

Financial Support: Research grant 2014/14494-8, São Paulo Research Foundation (FAPESP). IAJ was supported by Coordination for the Improvement of the Higher Education Personnel (CAPES); LNA was supported by a postdoctoral fellowship from Coordination for the Improvement of the Higher Education Personnel (CAPES), Programa Nacional de Pós Doutorado (PNPD)/CAPES 2015

P36 - Exosomes Containg tax Participate in the Inflammatory Process and serve as HAM/TSP Biomarker

Katia Kaori Otaguiri[1,2], Suellen Gomes Salustiano[1,2], Daiane Fernanda dos Santos[2,3], Evandra Strazza Rodrigues[3], Camila Cristina de Oliveira Menezes Bonaldo[2], Patricia Vianna Bonini Palma[2], Luciana Ambrósio[1], Fabíola Attié de Castro[1], Flávio Vieira Meirelles[4], Dimas Tadeu Covas[2,3], Juliano Coelho da Silveira[4], Simone Kashima[1,3]

1 Faculty of Pharmaceutical Sciences of Ribeirão Preto, Departament of Clinical, Toxicological and Bromatological Analysis, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

2 Regional Blood Center of Ribeirão Preto, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo Brazil

3 Faculty of Medicine of Ribeirão Preto, Department of Internal Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

4 Faculty of Animal Sciences and Food Engineering, Department of Veterinary Medicine, University of São Paulo, Pirassununga, São Paulo, Brazil

Background: Exosomes, a class of small membrane vesicles of endocytic origin, are secreted by most of the mammalian cells and participate in physiological and pathological processes, including inflammation and immune modulation. Aim: To test whether exosomes isolated from a tax-expressing cells could induce an inflammatory response and correlate to clinical findings observed in HTLV-1-infected patients. Methods: Exosomes from tax-expressing cells, were isolated by ultracentrifugation and characterized based on its content. Exosomes were cultured with non-infected PBMC for 72 hours and, at the end of this period, cytokines were quantified by intracellular staining by flow cytometry. At the same time, exosomes were isolated from serum of HTLV-1-infected individuals (asymptomatic carrier (AC) and HAM/TSP patients). The concentration of the exosomes and the presence of tax and hbz mRNAs were evaluated. Results: The exosomes isolated from C8166 cell line were positive for the presence of tax mRNA. After the incubation period of the exosomes with the non-infected PBMC, tax mRNA was detected in these cells, suggesting the transfer of tax mRNA from exosomes to the non-infected PBMC. We also observed, after 72 hours, that the proinflammatory cytokines were upregulated in T-cells after treatment with the tax-carrying exosomes compared to the negative control. The results suggest that the exosomes carrying tax may induce the production of proinflammatory cytokines and activate T cell immune response. Initial results of the clinical counterpart showed that exosomes isolated from HAM/TSP patients were positive for tax and hbz mRNAs while exosomes isolated from AC and healthy controls were negative for these mRNAs. Conclusion: These findings suggest that tax-containg exosomes have the potential to be used as biomarker of HAM/TSP. Moreover, the cytokine production by the

exosomes suggests the participation of these vesicles in the pathogenesis of the HAM/TSP by inducing the inflammatory features observed in this clinical picture.

Keywords: HTLV-1; HAM/TSP; tax; exosomes; immune response.

Financial Support: FAPESP (grant numbers 2015/21829-9, 2015/16985-1, 2014/22887-0, INCTC-2008/57.877-3, CTC-1998/14.247-6), CNPq (INCTC-573.754/2008-0) and CAPES.

P37 - Prospecting the transcriptome of infected mice cells with Mycobacterium tuberculosis for the validation of epigenetic markers related to protection in tuberculosis

Lima, F.T.; Frantz, F.G.

Tuberculosis (TB) is one of the leading causes of death from bacterial infection in the world today. In experimental TB, among the possible affected organs, the liver appears to present a very efficient response against infection when compared to the lung and spleen, presenting colony forming units counts of 1,000 to 10,000 times lower than in the lung. Thus, we hypothesized that in the liver, certain signaling pathways or effector mechanisms are quite efficient for the control of infections and could not be activated in the organs where the bacillus escapes from the immune system. The transcriptome of liver and lung macrophages was performed in the previous work of our research group, with the objective of identifying factors that differentiate the immune response in both organs, and that are related to the better efficacy of the liver in the presence of bacillus infection. Mechanisms of epigenetic control may be related to post-transcriptional changes that favor the growth of bacillus in the susceptible host or, on the other hand, favor the control of the disease in resistant individuals. Therefore, with this work, our objective is to analyze in silico the data already obtained from the transcriptome, in search of genes that encode epigenetic enzymes differentially expressed in the liver and lung. Once detected in silico which enzymes are associated with protection or susceptibility, the targets will be validated in vitro. Cells from the peripheral blood of healthy donors will be infected in vitro with M. tuberculosis and will be treated with inhibitors of the enzymes. After inhibition of these enzymes, the immune response and bacterial growth will be evaluated. Thus, we can identify epigenetic biomarkers involved in the response against TB in human cells, to suggest specific targets for therapeutic purposes.

Keywords: Tuberculosis, Epigenetic, Liver, Epigenetic enzymes, Transcriptome, M. tuberculosis.

Financial Support: Capes, FAPESP.

P38 - EVALUATION OF PLATELET ACTIVATION FACTOR RECEPTOR IN ORAL SQUAMOUS CARCINOMA CELLS CANCER IN VIVO AND IN VITRO

Tálita Pollyanna Moreira dos Santos[1], Leandro Figueiredo dos Santos[1], Cristiana Bernadelli Garcia[1], Lucas Oliveira Sousa[1], Luiz Carlos Conti de Freitas[2], Tarcília Aparecida da Silva[3], Andréia Machado Leopoldino[1]

1 - Department of Clinical Analyses, Toxicology and Food Sciences, School of Pharmaceutical Sciences of Ribeirao Preto, USP.

2 – Department of Oftalmology, Otohinolaryngology and Head and Neck Surgery, Medical School of Ribeirão Preto, USP.

3 – Department of Clinic, Pathology and Dental Surgery, Dental School of Universidade Federal de Minas Gerais, UFMG.

Background: Oral Squamous Cell Carcinoma (OSCC) has the highest incidence among oral cavity maligancies. Besides that, OSCC shows high mortality level and mantain 5-year overall survival rate. Under this scenario, explore its ethiopathogenesis can support the development of novel and effective therapeutic strategies against OSCC. Recently new oncogenics functions of the platelet-activating fator (PAF) and related PAF receptor (PAFR) ligands has been highlighted after anticancer therapies. However the mechanisms related to cell survival and resistance after a death stimulus are not fully determined. Objective: We investigate whether PAF-PAFR signaling axis impacts in Oral Squamous Cell Carcinoma (OSCC) progression and therapy response. Methods: The PAFR mRNA and protein levels were analyzed in a panel of head and neck squamous cell carcinoma (HNSCC) cell lines by real time PCR and Western blotting, respectively. The potential role of PAFR in modulating cell viability/survival was assessed by using a pharmacological inhibitor for PAFR (WEB 2086; SIGMA). Results: PAFR levels were similar among HNSCC cells. There was a 4-fold change up-regulation of expression in lysophosphatidylcholine acyltransferase (LPCAT) in a OSCC cell line (HN13). The PAFR antagonist (WEB 2086) reduced cell viability of two cell lines, a metastatic and a non metastatic. However other analysis (in vitro and in vivo) are in progress to explore the role of PAFR in OSCC tumor progression and therapy response.

Keywords: PAFR, oral cancer, cellular signaling.

Financial support: FAPESP, CAPES, CNPq.

P39 - Modulation of the intestinal microbiota in experimental colitis

Lia Vezenfard Barbosa[1], Jefferson Luiz da Silva[2], Camila Figueiredo Pinzan[2], Vânia Brazão Muniz[1], José Clóvis Do Prado Júnior[1], Cristina Ribeiro de Barros Cardoso [1,2]

1 University of São Paulo – Postgraduate Program in Biosciences Applied to Pharmaceutical Sciences

2 University of São Paulo – Postgraduate Program in Basic & Applied Immunology.

Intestinal disorders such as Inflammatory Bowel Disease result from an unregulated immune response, involving the intestinal microbiota and genetic susceptibility, that promotes chronic inflammation and extensive tissue destruction. Recent studies describe the effects of the hormone melatonin on inflammatory diseases, controlling immune responses. In addition to the action of melatonin on the circadian rhythm of humans, the molecule may also modulate the cycle of intestinal bacteria. Therefore, in the inflammatory context of experimental colitis, we aim to study the influence of melatonin on the modulation of experimental colitis, via intestinal microbiota. For this, C57BL/6 male mice will be exposed to DSS and treated with melatonin to verify their therapeutic potential in intestinal inflammation. Other animals will have the microbiota depleted and subsequent microbiota transference from animals previously treated with melatonin. The effects of the treatment and transference of the melatonin-conditioned microbiota in animals challenged with experimental colitis will be verified by analyzing the clinical evolution of the disease and the immunological investigation of intestinal, mesenteric and serum samples from the control and treated groups. The intestinal microbiota will be characterized by the extraction of DNA from the feces of the animals in experimentation. Identification of specific phyla by real-time PCR. Thus, using these approaches, we intend to understand the possible modulating action of melatonin on the intestinal microbiota in the experimental colitis model.

Keywords: Inflammatory Bowel Disease; Microbiota; Melatonin.

P40 - Expressão de Proteínas Recombinantes dos Vírus Dengue, Zika e Chikungunya para diagnóstico sorológico diferencial das viroses

PEREIRA, A. S.[1]; ARCHANGELO, B. S.[1]; TUCCI, L. F. F.[1]; RUSSO, E. M. S.[1]

1 School of Pharmaceutical Sciences of Ribeirão Preto - USP, Department of Clinical Analyses, Toxicology and Food Science

The arboviruses Dengue, Zika and Chikunguynia circulate simultaneously due to the common transmitter, vectors of class Aedes. These diseases are responsible for thousands of cases annually in Brazil with serious consequences to the population, leading to the death of many individuals.

These diseases have similar symptoms to each other, hindering the exact clinical diagnosis. Molecular tests are excellent, but unsuitable for mass diagnosis due to cost. Serological tests available on the market identify a single virus at a time and are of foreign production (90%), greatly increasing public health expenditures.

Because of all the risks of death or permanent sequelae that these viruses impose on individuals, a discriminatory diagnosis becomes necessary.

This project aims to produce recombinant proteins of the DEN-1, Zika and Chikungunya viruses that can be incorporated into rapid, discriminatory and unified diagnostic devices.

Sequences coding for the NS1 and EIII proteins of Dengue virus, NS1 from Zika and E2 from Chikungunya synthesized in vector pUC57 were isolated and amplified by PCR. The fragments of interest and the expression vector were digested by restriction enzymes and recovered from the agarose gel. After the binding reaction of the fragments to plasmid pET28a, the recombinant plasmids were transformed into DH10β competent for stock. The colonies were tested to confirm the presence of the insert and the construction of each expression plasmid was confirmed by nucleotide sequencing. Subsequently, the recombinant vector will be transformed into E. coli BL21(DE3)pLySs competent for expression of the protein to be induced by IPTG.

The protein will be purified by affinity chromatography and detected by SDS-PAGE and Western blotting.

These partial results show the first steps in the production in E. coli of some recombinant proteins that may be very important for the national production of serological tests to detect the target arboviruses of this project.

Keywords: Dengue; Zika; Chikungunya; Recombinant protein; Diagnostic

Financial Support: CAPES

P41 - Differential reconstitution of B-cell subsets in systemic sclerosis patients after autologous hematopoietic stem cell transplantation: initial results

João R Lima-Júnior[1,2], Lucas C M Arruda[1,3], Ana Júlia G Sousa[1,3], Daniela A Moraes[5], Dimas T Covas[1,5], Maria Carolina Oliveira[1,5], Kelen C R Malmegrim[1,4]

Authors' affiliations: 1 Program of Graduation on Biosciences Applied to Pharmacy, Department of Clinical, Toxicological and Bromatological Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil; 2 Center for Cell-based Therapy, Regional Blood Center of Ribeirão Preto, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil; 3 Program of Graduation on Basic and Applied Immunology, Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil; 4 Department of Clinical, Toxicological and Bromatological Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil; 5 Department Internal Medicine, Division of Clinical Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil.

Introduction: Autologous hematopoietic stem cell transplantation (AHSCT) is currently an effective alternative therapeutic approach for patients with severe systemic sclerosis (SSc), although its immune mechanisms are still not completely understood. Aim: To evaluate the reconstitution of naive, memory, regulatory and exhausted B-cell subsets in SS patients following AHSCT. Methods: Peripheral blood samples were harvested from fourteen diffuse SSc patients before transplantation and at 15, 30, 60, 120, 180 and 360 days post-AHSCT. The immunophenotype of B-cell subsets was assessed by flow cytometry. Results: Compared to baseline, the frequency and absolute counts of total CD19+ B-cells showed a significant decrease at 30 days, followed by an increase at 360 days post-AHSCT. The CD19+CD24hiCD38hi regulatory B cell (Breg; transitional B-cell subset) significantly increased in frequency and absolute counts at 360 days ($P<0.05$), while CD19+CD24hiCD27+ Breg (B10 subset) transiently decreased in absolute levels at 30 days ($P<0.01$). The CD19+CD38hi27hi Breg (plasmablast subset) increased at 15 days in frequency and absolute numbers ($P<0.01$). The frequency of CD20+CD43+CD27+CD69- (B-1 subset) increased at 30 days, followed by a decrease at 60 to 360 days ($P<0.05$), while no changes were detected in absolute numbers. Naïve B-cells significantly decreased in frequency and absolute counts at 30 days ($P<0.001$), followed by an increase at 360 days ($P<0.005$). There was a transient increase of non-class-switched memory-B-cells (CD19+CD27+IgD+) frequency at 30 days, followed by an increase to 360 days. The mature class-switched memory-B-cells (CD19+CD27+IgD-) frequency decreased at 120 to 180 days ($P<0.001$). No changes were detected in CD19+CD27highIgD- plasma-cells. The frequency of CD19+PD1+ exhausted B-cells increased at 30 days ($P<0.01$). Conclusion: SSc patients have increased Breg frequencies and/or absolute numbers after AHSCT, suggesting improvements of immunoregulatory mechanisms. Furthermore, following transplantation

SSc patients have decreased memory-B-cells and increased naive-B-cells values, which might contribute to self-tolerance reestablishment and disease remission on these patients.

Keywords: Systemic Sclerosis; Autologous Hematopoietic Stem Cell Transplantation; B cell; Regulatory B-cell; Cellular therapy.

Acknowledgements: Funding was provided by the Sao Paulo Research Foundation (FAPESP) and CAPES.

P42 - Evaluation of serum markers of inflammation, endothelial and platelet activation, and immunomodulation in sickle cell anemia patients treated with different therapeutic modalities

Keli Cristina de Lima (1), Júlia Teixeira Azevedo Cottas (2), Luciana Ribeiro Jarduli (1), Ana Cristina Silva Pinto (3), Belinda Pinto Simões (3,4), Kelen Cristina Malmegrim de Farias (3,5)

(1) Program of Graduation on Biosciences Applied to Pharmacy, Department of Clinical, Toxicological and Bromatological Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

(2) Program of Graduation on Basic and Applied Immunology, Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

(3) Department of Clinical, Toxicological and Bromatological Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

(4) Department of Internal Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

(5) Center for Cell-based Therapy, Regional Blood Center of Ribeirão Preto, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

Aims: Sickle cell anemia (SCA) is a genetic disease that results from a point mutation in the sixth codon of the β-globin gene, leading to deformation and decreased flexibility of red blood cells. Patients with SCA present a chronic inflammatory state. The therapeutic options of SCA therapy are: (i) chronic transfusion, (ii) hydroxyurea and (iii) allogeneic hematopoietic stem cell transplantation (HSCT), only the latter being a curative option. Although inflammatory processes are widely studied in SCA, there are few studies that compared the effect of different treatment modalities on serum biomarkers of inflammation, endothelial and platelet activation, and immunomodulation. This project aims to evaluate several serum biomarkers of SCA in the serum of healthy donors and SCA patients submitted to different therapeutic modalities. Methods: Peripheral blood samples were obtained from 15 afrodescendant healthy donors, 65 SCA patients (15 untreated, 15 treated with chronic transfusion, 15 treated with hydroxyurea and 20 SCA patients at one year post-HSCT. Serum biomarkers will be quantified by Luminex or ELISA methodology: VEGF, Endothelin-1, ICAM-1, E-selectin, P-selectin, CXCL4, HGF, FGF, TGF-beta (endothelial activation markers); Von Willebrand and thrombomodulin (platelet activation markers); pentraxin-3, IL-18, IL-6, IL-8, IL-2, IL-17A, TNF-alpha, INF-gamma, MCP-1, MIP-1b, IL-12p70, IL-33, IL-27, GM-CSF, CD163 (inflammation markers), PGE2, LT4 and IL-1B; Il-IL-10, BAFF APRIL, arginase-1, IL-10 (immunomodulatory markers). The quantification of nitric oxide and heme will be performed by colorimetric assays. Conclusions: So far, sample collection from all patients and healthy controls

has been carried. Serum aliquots were stored accordingly. Complete clinical data of the patients and controls were collected and analyzed for future correlation with laboratorial results.

Keywords: Sickle cell anemia; biomarkers; inflammation; immunomodulation; endothelial activation; platelet activation; allogeneic hematopoietic stem cell transplantation

Financial Support: CNPq and CAPES

P43 - Monoclonal antibodies and galectin 1 and 3 as possible diagnostic and therapeutic tools in Zika virus infection

Thaís P de Paula[1], Thalita B Riul[1], Fernanda U F Melo[2], Simone Kashima[2], Marcelo Dias-Baruffi[1]

1 Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brasil. 2 Hemocentro de Ribeirão Preto, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brasil.

The Zika vírus causes a major human viral disease of nowadays, and its pathophysiology isn't entirely known and there are no clinically vaccines or therapies approved capable of controlling and preventing its infections. The main symptoms are headache, hypersensitivity, itching and redness in the eyes, low fever, joint and muscle aches, skin rash and red spots, fatigue or exhaustion. The major concern within this virus is its connection with Guillain-Barré syndrome, a neurological disorder that can cause a permanent paralysis; and involvement of microcephaly in newborns of infected mothers, which may affect infant development. Zika vírus belongs to Flavivirus family, in which envelope protein is responsable for virus entry and represents an important target for neutralization by antibodies. Galectins 1 and 3, lectins recognizing β-galactoside linkers, whose recognition is preferably associated with the carbohydrate recognition domain (CRD), are involved in several biological processes, including the modulation of the inflammatory response and immunoregulatory activities, and a pathogen recognition capacity by galectin-1. Galectin-3 is not well related to viruses like galectin-1 does, altough this lectin is known to have effects on other infectious diseases. The production of compounds that help combat this virus represent important strategies for the development of tools for diagnosis and treatment of this public health problem. Polyclonal and monoclonal antibodies will be obtained by in vivo immunization of mice with the virus in suspension isolated from human serum and synthetic peptides of specific sequences for the zika virus and by fusion of spleen cells of these animals with myeloma cells. Characterizations will be made through SDS-PAGE electrophoresis, ELISA and immunoblots. We will investigate and evaluate galectins interactions with the zika virus, as well as their likely ability to reduce or inhibit infection.

Keywords: Zika virus, galectin-1, galectin-3, tools

Financial support: CAPES

P44 - Ageing is not associated with an altered immune response during Trypanosoma cruzi infection ageing and Trypanosoma cruzi infection

Rafaela Pravato Colato, Vânia Brazão, Fabricia Helena Santello, Míriam Paula Alonso Toldo, Gabriel Tavares do Vale, Carlos Renato Tirapelli, Gabriela Pereira-da-Silva, José Clóvis do Prado Jr

Introduction: Although numerous studies have examined the consequences of ageing, few have directly tested its effects during T. cruzi infection. Then, the aims of this work were to evaluate the influence of ageing and T. cruzi infection taking as tools several immune parameters.

Method: Male Wistar rats were infected with 1x105 blood trypomastigotes of the Y strain of T. cruzi and randomized into the groups: young control (YC), young infected (YI), senile control (SC) and senile infected (SI). Studies were performed on 09 and 16 days after infection. All spleen samples were processed for flow cytometric analysis, using conjugated-specific monoclonal antibodies and plasma was analyzed following instructions of a commercially available kit.

Results: Infected young animals displayed enhanced CD4+ T cells as compared to uninfected counterparts. Ageing also triggered a significant reduction in CD8+ T cells compared to young and uninfected groups. A significant decrease in MHC class II (RT1B) expression in all aged animals was observed, whether infected or not. The highest and significant levels of Thiobarbituric Acid Reactive Substances (TBARS) were noted in the aged and infected animals as compared to young-infected ones (16 day). Consequently superoxide dismutase (SOD) activity was reduced for both control and infected aged animals. Significant elevation of 8-isoprostane levels was found in aged control and infected animals. Plasma glutathione (GSH) concentration was reduced in aged control animals, as well as, in the young infected animals.

Conclusion: Our results demonstrate that the immune response in aged and infected animals was not worsened by the fact of animals being older. Ageing by itself compromised the immune response, and elevated reactive oxygen species, when compared to young counterparts, but it did not contribute to impairment of the immune response of T. cruzi infected and aged rats.

Keywords: Chagas' disease; Immunosenescence; Trypanosoma cruzi.

Financial support: FAPESP.

P45 - ANTIAPOPTOTIC AND IMMUNOMODULATORY EFFECTS OF GHRELIN IN CHRONIC CHAGAS DISEASE

Lessa, D.F.S; Silva. F.P.; Bronzon da Costa,C.M.; Abrahão, A.A.C.

School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP),São Paulo, Brazil.

This study evaluates the anti-apoptotic and immunomodulatoty effects of ghrelin in Wistar rats infected with the Y strain of T. cruzi. As experimental model for Chagas' disease infection it was used male young adult Wistar rats, intraperitoneally inoculated with 2x105 blood trypomastigotes of the Y strain of T. cruzi. Animals were randomized into six groups: untreated control (C); control treated with ghrelin (CG), infected (I), infected treated with ghrelin (IG), infected treated with benznidazole (IB); infected treated with ghrelin and benznidazole (IGB). Each experimental group consisted of 6 animals. Apoptosis assay was performed using spleen cells labelled with annexin V and propidium iodine for analyses early apoptosis, late apoptosis and viable cells percentage. NK and NKT cells was labelled with antibodies anti-CD161 (FITC) and anti-CD3+-allophycocyanin (APC) and counted by flow cytometer. Our data clearly demonstrated that ghrelin exerted an immunomodulation role as revealed by reduced percentages of NK and NKT cells as well as its anti-apoptotic effect triggering a reduction in early and late spleen cell apoptosis.

Keywords: Chagas Disease; Ghrelin; Anexin V.

Financial Support: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) (Proc. 2014/18682-3) and Coordenação de Aprimoramento de Pessoal de Nível Superior (CAPES).

P46 - Evaluation of the possible antiparasitic effects of chemical constituents obtained from Tithonia diversifolia, as well as its derivatives, in in vitro and in vivo models, in the infection by Leishmania amazonensis and Leishmania infantum

Naira F. Anchieta; Fernando B. da Costa, Sérgio de Albuquerque

The leishmaniasis are neglected tropical diseases and display a public health problem, reaching mainly poor and less favored regions. The cutaneous leishmaniasis (CL) caused by Leishmania amazonensis, is caracterized by ulcers with raised borders on the skin. The visceral leishmaniasis (VL), the severe form of the disease, is caused, among other species, by Leishmania infantum and exhibits a systemic mode. The current therapy of these diseases forms are not considered effective, for being higly toxic, with low efficiency, high cost, long treatment period and the emergence of cases of resistance to some used drugs. The current search for new compounds has focused on natural products, due to the low cost and high efficiency in several tests. Terpenoids have shown satisfactory effects in different infections, but mainly as anti-protozoa. The plant Tithonia diversifolia, belonging to the family Asteraceae, is rich in terpene-class compounds and has been extensively studied due to the anti-protozoal effects of its sesquiterpenes, but few studies have evaluated the diterpenes present in this plant. In this regard, our aim is to evaluate the action of the major chemical constituents found in Tithonia diversifolia in in vitro and in vivo models of L. amazonensis and L. infantum infections. For this, J774 cells will be infected with the parasites in vitro and the antileishmanicidal, cytotoxic and immunomodulation effects caused by these compounds will be evaluated. In in vivo assay, Balb/c mice will be infected and treated by gavage with the compounds using doses determined in in vitro assays. In the end of treatment, the animals will be euthanized and the parasitic load, immunological parameters and toxical effects will be analyzed. Thus, we hope that the present project may be able to provide new therapeutic alternatives for the treatment of LC and LV.

Keywords Leishmania amazonensis; Leishmania infantum; natural compounds; terpenoids; Tithonia diversifolia;

Financial Support:Fapesp; Capes, CNPq

P47 - Increasing tolerance to antimicrobial photodynamic treatment with New Methylene Blue in plant-pathogenic fungus Colletotrichum abscissum

Marcelo Ferreira da Cruz[1], Julia Cunha Gonzales[1], Guilherme Thomaz Pereira Brancini[1], Fernando Grine Martins[1], Mark Wainwright[2], Gilberto Úbida Leite Braga[1]

1 Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil

2 Liverpool John Moores University, Liverpool, United Kingdom

Aim: To investigate the development of resistance to antimicrobial photodynamic treatment (APDT) in plant-pathogenic fungus Colletotrichum abscissum. Methods: In a one-and-a-half-year-long experiment, we carried out seventy successive cycles of APDT of plant-pathogenic fungus Colletotrichum abscissum using the photosensitizer New Methylene Blue N (NMBN) combined with exposure to red light. The treated conidia of the fungus were then plated on potato dextrose agar medium. During subsequent incubation, the conidia borne on colonies from viable conidia (i.e., those which have survived the APDT) were pooled and submitted to the next cycle of treatment and selection. Results: We observed successive increases in conidia tolerance to APDT throughout the experiment. At the end of the 70th selection-round, conidial survival was 2.5 to 5 times (depending on the dose used) higher than that of the initial isolate. An increase in intracellular carotenoids was also observed throughout the successive cycles of conidial selection. Although the genetic basis of increased APDT tolerance and carotenoid production has yet to be determined, we believe that these two characteristics are correlated, since carotenoids quench singlet oxygen, the main reactive oxygen species produced during APDT with NMBN. Carotenoids in conidia have already been associated with increasing tolerance to APDT with phenothiazinium photosensitiziers in conidia of Neurospora crassa. Although somehow our results may cause concern to the APDT area, they cannot be considered completely unexpected, since there are other genera of plant-pathogenic fungi that are naturally resistant to photodynamic treatment. For instance, Cercospora species produces the potent photosensitizier cercosporin, which is used to damage the issues of the host plant, but are themselves resistant to its deleterious effects. Conclusions: For the conditions and fungal specie tested, we found development of resistance to APDT as increasing of conidial survival between the initial isolate and the isolate selected after 70 selection cycles.

Keywords: antimicrobial photodynamic treatment; Colletotrichum abscissum; resistance; increasing of conidial survival.

Financial Support: CAPES, FAPESP.

P48 - Evaluation of the effects of different biotherapics obtained from Trypanosoma cruzi on experimental Chagas disease

Danielle de Macedo Mano[1], Sérgio de Albuquerque[1]

1Laboratory of Parasitology, Department of Clinical Analysis, Toxicological and Bromatological, University of Sao Paulo, Ribeirao Preto, SP, Brazil

Aim: The aim of this study was to evaluate the effects of biotherapics of Trypanosoma cruzi on mice experimentally infected with Trypanosoma cruzi.

Methods: In a blind, controlled and randomized study, female of Balb/c mice were inoculated with 104 trypomastigotes forms of T. cruzi Y strain. The treatments were performed 5 days before and/or 48 h after infection in a pre- and post-infection treatment, respectively. The biotherapics were prepared with blood or heart obtained from animals in acute or chronic phase of the disease. The medicines were prepared according to Brazilian Homeopathic Pharmacopeia and Costa Living Nosodes methods. The biotherapics were administrated by ad libitum or by gavage. The following parameters were evaluated: infectivity, parasitaemia peak, survival and number of parasites present in the liver cells of the infected animals. Statistical analysis was conducted considering 5% of significance.

Results: All groups treated with biotheparics prepared with blood (in acute or chronic phase of the disease) had longer prepatent period and a lower number of trypomastigotes forms in the blood when compared with the control. However, no difference was showed when those groups were compared to each other. All groups treated with blood biotherapics showed larger reduction in the number of trypomastigotes forms in the blood during parasitaemia peak, when compared with the groups treated with heart biotherapics. The administration way interfered in the results. The gavage administration show better results than biotherapics administrated by ad libitum.

Conclusions: The results suggest that the effects of T. cruzi biotherapics depend on the raw material. The raw material had more influence than the preparing methods. Biotherapics of blood were able to reduce the number of parasites in the host blood. Homeopathic treatment of T. cruzi infection should be further investigated.

Keywords: Trypanosoma cruzi; Chagas disease; Homeopathy; Biotherapy

Financial Support: CAPES; CNPq

P49 - In vitro activities of phenothiazine dyes against Trypanosoma cruzi

Gisele Bulhões Portapilla[1]; Luiz Miguel Pereira[1]; Gilberto Úbida Leite Braga[1], Sérgio de Albuquerque[1]

1 Department of Clinical Analysis, Toxicology and food Science, FCFRP-USP, 14040 Ribeirão

Benznidazole and Nifurtimox are still the only antitrypanosomal medicines available to treat Trypanosoma cruzi infection more than 100 years after the Chagas' disease discovery. Both drugs display toxicity and low activity during the chronic phase of the disease. In this context, several classes of compounds have been studied to prospect molecules potentially active against T. cruzi. Phenothiazine dyes, especially methylene blue, represent a group of compounds very important for the treatment of malaria and have been described by activity against many pathogenic microorganisms and trypomastigote forms of T. cruzi. Therefore, the aim of this study was to evaluate the in vitro trypanocidal activity from four commercial compounds phenothiazines (methylene blue, new methylene blue, toluidine blue and 1,9-dimethyl methylene blue) and four synthetic phenothiazines (DO15, DO16, DO37 and DO43). Cytotoxic activity of these dyes was determined in LLCMK2 mammalian cells and the biological assays evaluated against amastigote forms of T. cruzi (Tulahuen strain) from colorimetric methods in vitro. All of them showed low cytotoxicity and higher trypanocidal activity than Benzonidazol (standard drug). Considering the relationship between cytotoxicity and trypanocidal activity (selectivity index, SI), all of them showed SI ≥ 10 as recommended for trypanocidal drugs screening. Methylene blue is already a drug used in humans and has a low cost and toxicity. Therefore, the results may contribute to development of alternative therapies for the treatment of Chagas' disease.

Keywords: Chagas' disease, Treatment, Phenothiazine dyes, in vitro assay

Financial Support: CAPES

P50 - Umbilical cord derived-mesenchymal stromal cells conditioned with hypoxia or IL-17 for treatment of experimental colitis

Nádia de Cássia Noronha[1,4], Amanda Mizukami[4], Maristela D Orellana[4], Dimas Tadeu Covas[4], Cristina Ribeiro B Cardoso[3,4], Kamilla Swiech[3,4], Kelen C R Malmegrim de Farias[3,4]

1 Program of Graduation on Biosciences Applied to Pharmacy, Department of Clinical, Toxicological and Bromatological Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

2 Program of Graduation on Basic and Applied Immunology, Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

3 Department of Clinical, Toxicological and Bromatological Analysis, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

4 Center for Cell-based Therapy, Regional Blood Center of Ribeirão Preto, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

Mesenchymal stromal cells (MSC) constitute a heterogeneous cell population widely distributed in adult tissues. MSC have in vitro and in vivo immunomodulatory and regenerative properties. They have been currently used for therapy of inflammatory, immunological and degenerative disorders. However, some limitations, such as cell senescence by excessive in vitro expansion, reduction or inconsistency of the therapeutic potential and low survival of transplanted cells, require immediate search for new approaches to produce robust and functional MSC for therapies. There is a need for bioprocesses optimization in order to generate cells with regenerative or immunodulatory properties. Aims: To evaluate the function and therapeutical potential of MSCs isolated from umbilical cord (hUC-MSC) and conditioned with hypoxia and/or interleukin-17; and to establish a scalable bioprocess to expand hUC-MSC for therapy under xenoantigen-free conditions. Methods: hUC-MSCs will be isolated, expanded in stirred-tank bioreactor coupled to microcarriers and conditioned with hypoxia and/or IL-17. Subsequently, MSC will be characterized by: i, immunophenotyping and differentiation assays; ii, in vitro immunossupresisve function (inhibition of T cell proliferation) and regenerative (angiogenic potential in matrigel membrane assay) functions; iii, in vivo immunomodulatory and/or regenerative potential, in experimental models of acute or chronic colitis induced by sodium dextran sulfate (DSS). Preliminary results: Experiments performed with different microcarriers demonstrated that Cultispher (porcine collagen) exhibited good cell adhesion, expansion and recovery, HyQSphere (polystyrene) exhibited good cell adhesion in short time period of expansion and SphereCol (human collagen coated) presented excellent rate of cellular recovery. hUC-MSC expanded on SphereCol microcarries by 6-hour adhesion phase and intermittent shaking, showed intense cell expansion (fold expansion of 11.7). MSC cultured on SphereCol presented excellent rate of cellular recovery once they are coated with human collagen layer, which is easily

degraded by enzymatic digestion. Conclusions: SphereCol microcarriers accomplished the xenofree culture conditions, exhibited excellent expansion rate of hUC-MSCs and was selected for subsequent evaluations.

Keywords: mesenchymal stromal cells, cell therapy, immunomodulation, hypoxia

Financial Support: CAPES