Quimioterapia-Chemotherapy

QT01 - Cure control of Trypanosoma cruzi infected schoolchildren from Berilo, Jequitinhonha Valley, MG, Brazil, together with genetic characterization of previous isolated parasites from children and respective mothers.

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The treatment of the *Trypanosoma cruzi* infection is more successful in younger age groups and there is explicit WHO indication for childhood and adolescence. Thus, six seropositive children identified in a serological inquiry in the municipality of Berilo, Jequitinhonha Valley, MG, Brazil, were treated with Benznidazole. The cure control was performed together with genetic characterization of *T. cruzi* strains isolated from these children and their respective mothers. The cure control was performed through parasitological tests (Hemoculture and PCR), conventional (ELISA and IHA) and non conventional (AATV) serology and clinical examination including ECG, Thorax X-ray and Echocardiogram. After a six month treatment all children presented reactive serology and PCR. Two cases had positive hemoculture showing an evident therapeutic failure. Despite the lack of medical indication, these two children were treated with Nifurtimox. One of them remained with discrete ECG alteration already observed before treatment. Genetic characterization of the *T. cruzi* strains (from the children and their mothers) was carried out by rRNA 24 Sα, mitochondrial gene of coxigenase (CO) and microsatellite techniques. All samples belong to rRNA group type I (amplicon 125pb), haplotype C for coxigenase gene, being both of them *T. cruzi* II, monoclonal and with identical microsatellite profiles to the respective mother suggesting congenital transmission. Children were reevaluated 18 and six months after treatment with benznidazol and nifurtimox, respectively. At that time all children presented reactive conventional serology, negative hemoculture and one of them, was again positive PCR. In the clinical reevaluation all children remained in the indeterminate form of the infection, except one child that presented the same ECG alteration (increased Pr interval), now with a biventricular dilated myocardiopathy detected through Echocardiography. These evaluations were not conclusive neither for parasitological nor for clinical impact. New evaluations will be carried out annually. **Financial support: FAPEMIG, CNPq and PROEX/UFOP.**

QT02 - EARLY DEFINITION OF CURE CONTROL OF EXPERIMENTAL ACUTE PHASE OF CHAGAS DISEASE IN MURINE MODEL

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The demonstration of treatment efficacy in Chagas disease is very complex due difficulties in establishment of cure criteria and the limitations of the methodologies used. Experimental studies in mice are useful to test different chemotherapy schedules and drug efficacy. The combination of different methodologies is necessary and different approaches are employed. To define early post-therapeutic response in BALB/c mice infected with *T. cruzi* stocks from different major genotypes in single and mixed infections, biological samples were assayed by five methods including parasitological (fresh blood examination-FBE, hemoculture-HE and PCR) and serological (ELISA and detection of anti-live trypomastigote antibodies by flow-cytometry-FC-ALTA). Animals were treated with 100mg of Benznidazole/Kg of body weight/20 consecutive days starting in 10th and pos-treatment classified as treated not cured(TNC), treated cured(TC) and dissociated(DIS). Therapeutic failure was detected in 72.2% of treated animals, which were considered TNC. In the TNC group the FBE was able to detect parasitemia only in 19.8% of TNC animals. On the other hand, 79.3% and 93.0% of therapeutic failure were detected by hemoculture and PCR, respectively. Most TNC animals with negative hemocultures was positive PCR(27/29). Animals with positive PCR, but with negative hemoculture, also presented positive serological tests. FC-ALTA was more efficient in the accurate categorization of the different groups of mice in relation to treatment efficacy than ELISA becoming negative only three months after treatment. Strong correlation was observed between FC-ALTA and PCR. Despite the antibodies levels observed in (ELISA) and (FC-ALTA) for TNC(HE+) and TNC(HE-) were not significantly different, TNC(HE-) group showed always lower reactivity in comparison to TNC(HE+). Data confirmed the general idea that higher parasitemia and consequently more antigenic stimulation leads to higher antibody production. Thus, for cure criteria, HE associated to PCR/FC-ALTA is outstanding approaches for early definition of post-therapeutical efficacy in mice.**Supported by: FAPEMIG, CNPq and UFOP.**

QT03 - ANTI-TRYPANOSOMAL ACTIVITY OF EXTRACTS, FRACTIONS, AND SUBFRACTION OBTAINED FROM *Anthemis tinctoria* in *Trypanosoma cruzi*

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Trypanosoma cruzi is the etiologic agent of the Chagas' disease, endemic in many countries of the Central and South America, including Brazil. This disease still constitutes a serious medical and social problem, affecting 18 million people and causing disease in 45,000 patients annually. So, it is important to obtain new potent drugs with low side effects. It is considered that 25% of all the modern medicines are derived direct or indirectly from plants. The species of Anthemis are used widely in the pharmaceutical industry of cosmetics and foods. In the traditional medicine, this plant is used in the hepatic inadequacy and jaundice treatment and your flowers have known properties that are used as antiseptic. In the present work we investigated the effect of crude extract, fractions, and subfraction containing sesquiterpene mixture obtained of flowers from *Anthemis tinctoria* in *T. cruzi*. Epimastigotes were inhibited when treated with aqueous crude extract, dichlorometane fraction, and subfraction presenting IC50 of 2.3 μg/ml, 1.8 μg/ml, and 1.5 μg/ml, respectively. The sesquiterpene mixture was able to inhibit the interaction of *T. cruzi* with LLCMK2 cells at concentration of 0.5 μg/ml (rate above 50%). For the investigation of the cytotoxic effect LLCMK2 cells were treated with the subfraction, which CC50 was 7.0 μg/ml (SI = 4.67). Epimastigotes treated with subfraction showed ultrastructural and morphologic alterations like rounded cells, formation of blebs in the citoplasmatic membrane and flagellum, observed by transmission and scanning electronic microscopy. These results show a good in vitro activity of *A. tinctoria* in *T. cruzi* probably associated to the effect in the cellular membrane of the parasite. In addition, natural products can be a source of new drugs with antiprotozoan activity and low toxicity.

QT04 - In vitro screening for antileishmanial and trypanocidal activity of Brazilian Cerrado plants.

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The side effects and the emerging resistance to the available drugs against leishmaniasis and trypanosomiasis lead to the urgent searching for new therapeutic agents against these diseases. Screening natural products provide the chance to discover new molecules highly effective. Thus, sixty organic solvent extracts of seventeen medicinal plants from the Brazilian Cerrado (a savannah-like vegetation) were therefore evaluated *in vitro* for their antiprotozoal activity against epimastigotes of *T. cruzi* (Y strain) and promastigotes of *Leishmania braziliensis* (2904 strain) and *L. chagasi* (PP75 strain). For the initial screening culture forms ($5x10^6$ cells/ml) of LIT and Schneider medium were incubated in 96 well plates at 26^{o}C with 1,000 and 100 μg/ml of the natural extracts. After 48h incubation the antiparasitic effect was observed in an inverted microscopy Olympus and the active extracts were incubated with the parasites at serial dilutions for determining of the IC_{50} value, by hemocytometer counting (*T. cruzi*) and colorimetric MTT assay (*Leishmania*). As control were used amphotericin B(1μM), Benznidazol (50μg/ml) and DMSO 1%. The citotoxicity (CC_{50}) of active extracts was evaluated in Vero cells ($2x10^4$ cells/well) by the MTT method. Among the analyzed plants, only *Annona coreacea* (Anonaceae), *Spiranthera odoratissima* (Rutaceae) and *Pterodon* sp. (Fabaceae) were active against *T. cruzi* (IC_{50}= 23.8, 44.7 and 181.1μg/ml, respectively). With exception of *S. odoratissima* (CC_{50}= 148.5μg/ml) all active extracts showed a high citotoxicity (10-37μg/ml). Twenty-three percent of the test extract showed some leishmanicidal effect, particularly those of *Calophyllum brasiliense* (Clusiaceae), *S. odoratissima* (Rutaceae) and *Annona dioica* (Annonaceae) with IC_{50} values between 10 to 41μg/ml. The antileishmanial effect of these extracts was at least three times higher than the citotoxicity. Fractionations of effective extracts are under progress to identify the active antiprotozoal compounds. Supported by: CNPq, UFSC, UNIVALI

QT05 - IN VITRO SUSCEPTIBILITY OF PLASMODIUM FALCIPARUM TO STANDARDIZED INFUSIONS (TEAS) OF ARTEMISIA ANNUA CULTIVATED IN CAMPINAS, SÃO PAULO.

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Introduction: Worldwide occurrence of malaria has reached about 300 to 500 million cases annually in recent years. One of the major problems associated with the resurgence of malaria is the resistance which parasites have acquired to clinically used drugs.Artemisia annua L. is a plant of Chinese origin which has been used for the treatment of fever

and malaria for more than 2,000 years. The antimalarial substance artemisinin is isolated from the leaves of this plant. Artemisinin is important in the treatment of multi-drug resistant strains of Plasmodium falciparum. Objective: Evaluate in vitro susceptibility of standard P. falciparum K1 and Dd2 strains to standardized leaf infusion (tea) of A. annua, cultivated at UNICAMP (Campinas, São Paulo). Result: Standard A. annua L. infusion (tea) prepared from leaves (5 g) in 100 ^{o}C water (1 L) presented 100 % inhibition of blood forms of P. falciparum, as did dilutions of 1:10 to 1:1,250 of this tea. More dilute infusion (1:6,250 to 1:15,000) permitted observance of gradual reduction of inhibitory power in these strains. Conclusion: Standard A. annua L. infusion (tea) presented significant inhibitory activity to standard P. falciparum strains K1 e Dd2, even at high dilution. There are reports in the literature that compounds present in A. annua act synergistically with artemisinin thus potentiating the action of this active principle in vitro. Assays involving isolated field strains of P. falciparum from the Brazilian Amazon are underway to evaluate the susceptibility of these strains to A. annua infusions. The implementation of more sensitive and rapid methods will be necessary to confirm the levels of inhibition found and to guarantee the reproducibility of the result. Keywords: Plasmodium falciparum, Artemisia annua L., Amazon.

QT06 - Evaluation of the in vitro activity of Ethanolic Extract and some compounds isolated from *Combretum leprosum* against *L. amazonensis* promastigotes

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Leishmania amazonensis causes human diseases that range from self-healing cutaneous lesions to diffusion cutaneous leishmaniasis. The chemotherapeutic of this disease require long-term treatment and it has been based on the use of pentavalent antimonials. In this study, we tested the ethanolic extract (EEFr) and four different compounds (CLEFT-01 and its modified derivates CLEFT-04, CLEFT-06 and CLEFT-07) isolated from the fruits of the plant *Combretum leprosum* to determine their in vitro antileishmanial effect against *L. amazonensis* promastigotes. Briefly, promastigotes parasites were treated in the absence or in the presence of several concentrations of the extract and its derivates compounds for 5 days. The parasite numbers were daily scored at Neubauer chamber with erythrosin B. Our results demonstrate that CLEFT-01 is potent in inhibiting promastigotes growth with IC50 3,3μg/ml. The derivates compounds CLEFT-06 showed IC50 3,48μg/ml and CLEFT-07 showed less activity with IC50 value = 5,9μg/ml. CLEFT-04 did not showed effect against the parasite. The EEFr presented a dose-dependent inhibitory effect. The reversibility of the inhibitory effect on promastigotes growth was tested by incubating parasites with the drugs up to 72 hours and after that period the parasites were washed and ressuspended in fresh medium. The action of the isolated compounds was not reverted. The parasite growth remained reduced in 70-90% even after the suspension of the drug. It was also showed that the EEFr (12,5μg/ml) and the four different compounds (5μg/ml) were able of inhibit promastigotes growth in the log phase of the division. The results demonstrate that CLEFT-01, CLEFT-06 and EEFr showed a high inhibition of the growth in the log phase of 49%, 36% and 29% respectively. These results contribute for the advance in the research for new anti-leishmania drugs, and suggest that CLEFT-01 a compound isolated from *C. leprosum* have promising antileishmanial potential. Financial Support: CAPES, CNPq

QT07 - Activity of Ethanolic Extract and some compounds isolated from *Combretum leprosum* in the infectivity and intracellular development of *Leishmania amazonensis* in murine macrophages

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Although considerable advances have been made in recent years, the chemotherapy of leishmaniasis is still based on pentavalent antimonials, diaminas and antifungal polyene. Most of these agents generate resistance, require long-term treatment and present a considerable toxicity. Plants are very rich sources of new compounds that can be developed into new drugs for various diseases. In this work, we used the ethanolic extract (EEFr) and four different compounds (CLEFT-01 and its modified derivates CLEFT-04, CLEFT-06 and CLEFT-07) obtained from the fruits of the plant *Combretum leprosum* to determine their in vitro action in the *L. amazonensis* amastigotes survival inside murine macrofages. In all experiments were used IC80 previously established of 5μg/ml to the four different compounds and 12,5μg/ml to EEFr. CLEFT-01 and CLEFT-06 showed inhibitory activity of 90% and 89% respectively in the differentiation process of lesion-derived amastigotes to promastigotes. EEFr and CLEFT-07 showed inhibition of 24% and 33% respectively. The number of lesion-derived amastigotes was scored daily by counting with Erythrosin B. To evaluate the effect of these compounds on intracellular development of *L. amazoensis*, promastigotes from early stationary phase were used to infect mouse peritoneal macrophages and after that, the cultures of infected macrophages were treated with theses drugs for 24, 48, 72 and 96h. The infection rate and the number of intracellular parasites decreased at a time-dependent way in infected macrophages treated with EEFr and with all compounds derivated of *C. leprosum.* The CLEFT-01 showed the highest antileishmanial activity, reducing the number of intracellular

amastigotes in about 84% after 96h of treatment. We also had tested citotoxicity against mammalian cells for all the drugs, but none showed effect in murine macrofages. These results suggest that CLEFT-01 has promising antileishmanial potential and contribute for the advance in the research for new chemotherapy for Leishmaniasis. Financial Support: CAPES, CNPq

QT08 - Activity of semi-purified fractions of three *Baccharis* species against *Leishmania (Leishmania) amazonensis*

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Introduction and objective. Many drugs emerged from popular knowledge such as artemisine, aspirin and quinine, originally isolated from plants. *Baccharis* species are used in popular medicine against gastrointestinal disorders and inflammatory processes. Experimental protocols, showed that extract of some *Baccharis* species had good response against *T.cruzi* and bacteria strains, but there are no studies against *Leishmania* sp. The aim of this work was evaluate anti-leishmanial activity of three *Baccharis* species. **Methods.** The leaves of *B. microdonta, B. regnellii* and *B. uncinella* were collected in Campos do Jordão, São Paulo State. They were dried and powdered, extracted exhaustively with methanol and partitioned with hexane (HEX), dichloromethane (DCM) and ethyl acetate (AcOEt). The semi-fractions were evaluated against promastigote forms of *L.(L.)amazonensis* in a concentrations of 6.0 to 100.0μg/well and after 24h the survival rate was evaluated using a Neubauer chamber. The J774 macrophages were used to evaluate the cytotoxicity of *B. regnellii* (DCM) semi-purified fraction (6.00 to 100μg/well). **Results and conclusion.** The main extract with anti-leishmanial activity was *B. regnellii* (DCM) with inhibitory concentration 50 (IC50) of 12.0μg/well, followed by *B. uncinella* (HEX), with an IC50 of 20μg/well and the promastigote forms treated with *B. uncinella* (AcOEt) had the motility affected. The others semi-fraction showed no anti-leishmanial activity. The cytotoxic assay showed that *B. regnellii* (DCM) was toxic in the highest concentrations used (100.00 and 50.00μg/well) with an IC50 of 60.0μg/well. The leaves from *B. regnellii* and *B. uncinella* have different compound, but they were capable of inhibit the growth rate of promastigote forms killing or decreasing its motility (may be a leishmaniostatic activity). Supported by LIM-50

QT09 - Antitrypanosomal Activity of Biochanina A, an Isoflavone Isolated from *Cassia fistula*

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Introduction.Protozoan diseases constitute the most widespread global health problem. American Tripanosomiasis is an infectious disease caused by the protozoan parasite *Trypanosoma cruzi* Chagas disease is a major cause of morbidity and mortality in many regions of South America. No effective drug exists and benznidazole is believed to be ineffective and too toxic for treating chronic infections. Plants have always represented the ultimate challenge for synthetic chemists, supplying novel secondary metabolites, contributing with several antitrypanosomals. *Cassia fistula* (Leguminosae) has been traditionally used in Brazil as febrifuge, diuretic and recently reported as an adjuvant in the treatment of Malaria. **Objectives**. To isolate and test the active metabolite from the fruits of *C. fistula* against *T. cruzi*. **Results**. The Nuclear Magnetic Resonance Spectroscopy (NMR) studies of the isolated compound from *C. fistula* fruits revealed an isoflavone metabolite, identified as biochanina A. Using *in vitro* assays, it was tested against LLC-MK2-derived trypomastigotes of *T. cruzi* (Y strain) and presented a 50% Effective Concentration of 18.32 microg/mL, as determined by the MTT assay. In order to evaluate the mammalian cytotoxicity, it was tested in mouse peritoneal macrophages, and showed a 50% Effective Concentration of 42.58 microg/mL after 48 h incubation by MTT assay. **Conclusion**. These results indicate that *Cassia fistula* presents a secondary metabolite active against *T. cruzi* trypomastigotes and if adequately studied, biochanina A could be used as a tool in the design of novel drug prototypes against Chagas disease. This work was supported by FAPESP

QT10 - EFFECTS OF TWO NEOLIGNANS DERIVED FROM BRAZILIAN PLANTS ON GROWTH AND ULTRASTRUCTURE OF EPIMASTIGOTES OF *Trypanosoma cruzi.*

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Chemotherapy of Chagas disease is based on the only two available drugs that are the nitrofuran nifurtimox (Bayer,

recently discontinued) and the nitroimidazol benznidazol (Roche). These drugs have significant activity only in the acute and recent chronic phases of the disease, and its efficacy varies among different strains of *Trypanosoma cruzi*. Lignoids are widely distributed natural plant compounds and have been studied as potencial drugs against several parasites as *T. cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. We tested two neolignans from Brazilian Lauraceae (Licarin A e Burchellin) on epimastigote forms and intracellular amastigote forms of *T. cruzi*. The parasites were strongly affected by all concentrations tested of both compounds. Both drugs at 100 uM caused the death of all parasites. Epimastigotes after 24 hours of treatment with 100 uM Licarin A presented swollen mitochondrion that in most cases occupied most part of the cytoplasm and lost the organization of the internal mitochondrial cristae. Some treated parasites presented condensed and disorganized kDNA. Frequently nuclear chromatin seemed also disorganized. The perinuclear membrane was lose and in many regions there was a complete disconnection of the two layers. Most treated parasites presented a complete cytoplasmic disorganization with alteration of several organelles, including Golgi complex. Several epimastigotes presented signs of cell lysis. Parasites treated with Burchellin at a concentration of 50 uM presented cellular disorganization and organelle alterations after 72 hours. Treated parasites presented autophagosomes that occupied almost completely the cytoplasm. Several parasites presented perinuclear spaces and chromatin disorganization. Many treated parasites presented cell lysis features. Financial support: FAPERJ, PAPES IV (Fiocruz / CNPq), European Commission, Programa de Núcleos de Excelência (PRONEX), Financiadora de Estudos e Projetos (FINEP).

QT11 - EFFECT OF YANGAMBIN LIGNAN FROM *Ocotea duckei* Vattimo (Lauraceae) ON ULTRASTRUCTURE OF *Leishmania* PROMASTIGOTES

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Chemotherapy for leishmaniasis is still unsatisfactory due to toxicity and limited effectiveness of the available drugs. These drawbacks demonstrate the urgent need of new therapeutic agents for the treatment of leishmaniasis. Several compounds isolated from plants have already been identified as antileishmanial agents, a fact showing that plants yet to be investigated represent an important source of new drugs against parasitoses. Yangambin, a lignan obtained from *Ocotea duckei* Vattimo (Lauraceae) shows antileishmanial activity, in vitro, on promastigote forms of *Leishmania chagasi* and *Leishmania amazonensis*. The aim of this study was to analize, at the ultrastrutural level and under confocal microscopy, the in vitro effects of yangambin against the protozoans *L. chagasi* and *L. amazonensis*. The results showed that treatment of *Leishmania* promastigotes with corresponding IC_50 /72 h value of yangambin induced a significant number alterations in both studies. Promastigotes of *L. chagasi* and *L. amazonensis* dyed with acridine orange (20 μg/mL) in the presence of yangambin showed alterations as reduction in the emission of green fluorescence, increase of red fluorescence, changes in cell morphology as abnormally shaped body, rounded forms cells and motility reduction in relation to the control. Picnotic nuclei were observed in several cells of both parasites culture. Ultrastructural analysis of promatigotes treated with yangambin showed electronluscent cytoplasm, reduction of endoplasmic reticulum profile, marked mitochondrial swelling with increase in number of mitochondrial cristae, parasites with multiples nucleuses, formation of vesicles which resembles apoptotic bodies and lipid deposition in mitochondrial matrix. These alterations (in both analysis) were suggestive of programmed cell death (apoptosis and/or autophagy). In conclusion all these effects together could ultimately result in general ultrastructural disorganization which eventually impair cell viability and lead to parasite death. Our results points towards the potential use of yangambin as a chemotherapic agent for leishmaniasis.

QT12 - Activity of Methanolic Extracts of *Potomorphe umbellata* and *Bixa orellana* in the amastigotes forms of *Leishmania amazonensis*

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The control of leishmaniasis constitutes a serious public health problem, with difficult treatment and several side-effects due to the high toxicity of the clinically used drugs. In the last years, many researches have been developed to evaluate the efficiency and security of the use of medicinal plants for prevention and treatment of diseases, including leishmaniasis. The methanolic extracts of *Potomorphe umbellata* and *Bixa orellana* were tested in our laboratory and demonstrated a significant activity against promastigote forms of *L. amazonensis*, which has been associated with all clinical forms of leishmaniasis. So, in this work we tested these extracts to determine their in vitro antiparasitic effect against amastigotes of *L. amazonensis*. For antiamastigote activity, 5 x 10^5 J774 A1 cells/well were cultured in 24 chambers TPP slides in RPMI-1640 medium. Once macrophages were adhered, 5x1010^6 *L. amazonensis*promastigotes/well were added and maintained at 33^{o}C in 5% CO2 for 3 hours. Non-internalized promastigotes were eliminated and dilutions of

extracts were added and maintained at 37^{o}C in 5% CO_2 for 24, 48 and 72 hours. At last 500 macrophages were counted per well for calculating % infected macrophages and amastigotes per macrophages. None of extracts tested induced an expressive reduction of intracellular amastigotes. Others extracts of medicinal plants are being tested against *Leishmania*.
Supported by FAPEMIG and UFJF.

QT13 - Anti-Leishmanial Effect of Bixin, a carotenoid purified from *Bixa orellana*

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Leishmaniasis is a group of diseases with a significant cause of morbidity and mortality in several countries. Leishmaniasis threatens 350 million people worldwide and an estimated 2 million new cases occur annually. The current treatment for leishmaniasis presents high toxicity and is not fully effective. The lack of an effective antileishmanial drug has caused renewed interest in the study of plants as source of new chemotherapy. Bixin is a structural unusual carotenoid easily isolated from *Bixa orellana* seeds by acid-base extraction. This product is now largely used as a natural coloring agent in foods due to its accessibility and lack of toxicity. Some studies have indicated that the consumption of carotenoids is associated with a lower incidence of cancer and cardiovascular diseases. In this work, we studied the activity of bixin against promastigotes and amastigotes forms of *Leishmania amazonensis*, the causative agent of cutaneous and diffuse cutaneous leishmaniasis. Bixin on different concentrations was tested on promastigotes cultures and on macrophages infected with amastigotes forms. Bixin showed a significant activity against promastigotes, after 24 hour treatment, with 70% inhibition at a concentration of 50 uM. Furthermore, bixin reduced the amastigote survival to 20% at 50 uM. At 100 uM, bixin did not show cytotoxic effect on peritoneal macrophages as evaluated by trypan blue, after 24 hour of treatment. In order to identify the possible mechanism involved on anti-amastigotes activity we analyzed the NO production of macrophages. Our results showed a dose-dependent increase in the NO production. A direct effect on parasite as well as stimulation of macrophage NO production may be involved on bixin leishmanicidal activity. The cell cycle of parasites incubated or not with bixin is being analyzed. These results provide new perspectives for a novel compound with leishmanicidal effects obtained from natural products.
Supported by CNPq, Capes-PROCAD e FAPERJ.

QT14 - ULTRASTRUCTURAL ANALYSIS OF *Trypanosoma cruzi* EPIMASTIGOTES AND TRYPOMASTIGOTES TREATED WITH GERANYLGERANIOL

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Pterodon pubescens Benth. (Leguminosae), known as sucupira branca, is a native tree species easily found in all over the central region of Brazil and its seeds are used in folk medicine for their antirheumatic, analgesic and anti-inflammatory properties. In a previous work, fractions were obtained from the ethanol extract of *P. pubescens* seeds by sequential extraction (hexane/dicholormethane/ethyl acetate), and was further separated by HPLC, consisting one of those fractions, geranylgeraniol (GG-OH) (Silva et al., 2004). GG-OH was active against bloodstream trypomastigotes, also inhibiting the epimastigote and intracellular amastigote proliferation (Menna-Barreto et al., 2006). In the present work, we extend the investigation of mechanism of action of GG-OH, employing transmission electron microscope technique. TEM analysis of GG-OH-treated epimastigotes and trypomastigotes showed mitochondrial swelling with the formation of concentric membranar structures inside the organelle, the presence of mielin figures on cytosol, as well as endoplasmic reticulum profiles surrounding organelles. Treated epimastigotes also presented an increase in the number of lipid inclusions, while GG-OH induced an important disruption of kDNA network in trypomastigotes. Flow cytometry analysis confirmed mitochondrial damage in treated epimastigotes and trypomastigotes as well as an increase in the lipid content in epimastigotes. These findings encourage us to further investigate the possible interference of GG-OH with the lipid metabolism in *T. cruzi*.

QT15 - QUERCITRIN: THE MAIN ACTIVE COMPONENT OF THE AQUEOUS EXTRACT OF KALANCHOE PINNATA IN CUTANEOUS LEISHMANIASIS

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Previous studies on the antileishmanial properties of Kalanchoe pinnata led to the demonstration of its in vitro activity on intracellular parasites as well as its efficacy in orally treating cutaneous leishmaniasis of both mice and human.

The present study aimed at identifying the antileishmanial compounds of aqueous extract using anti-Leishmania amazonensis amastigote activity-guided fractionation. The bioguided fractionation led to the separation of an active flavonoid fraction followed by the isolation of quercitrin, a flavonoid with strong antileishmanial activity. Quercitrin was more active in vitro than the current drug Pentostam(IC50 4 ug/ml and 20 ug/ml, respectively). No inespecific citotoxicity was observed with concentrations bellow 10 ug/ml.Four additional flavonoids were isolated from the same active fraction: kapinnatoside, quercetin-arabino-rhamnoside, afzelin and an glucosyl-flavone, but none was superior to quercitrin when tested on leishmania-infected macrophages. When administered by the oral route to Leishmania amazonensis-infected mice, quercitrin was shown to be as potent as the K. pinnata aqueous extract in inhibiting the lesion growth. Metabolism study in mice orally given quercitrin and quercetin corroborates with the literature data indicating that glucuronides are the main plasma metabolites of this class of molecules. This study has identified the flavonoid quercitrin as the main active component of the aqueous extract of K. pinnata in cutaneous leishmaniasis. Financial support: CAPES, FAPERJ

QT16 - *In vitro* evaluation of leishmanicidal and trypanocidal activity of crude extracts and isolated compounds from *Solidago chilensis* and *Laurencia* species.

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Leishmaniasis and Chagas' disease are major public health problems with a limited number of drugs for treatment. Plants represent a valuable source of prototype molecules for the development of new chemotherapy agents. The aim of this study was to evaluate antiprotozoal activity of extracts from roots of *Solidago chilensis* Meyen and from two *Laurencia* species (*Laurencia flagelifera* J. Agardh, *L. microcladia* Kutz) and the pure compounds Solidagenone, isolated from *S. chilensis* and Elatol, isolated from *L. microcladia*. Crude extracts and isolated compounds were solubilized in DMSO and tested against promastigotes of *Leishmania amazonensis* (575 strain), *L. chagasi* (PP75 strain) and epimastigotes of *Trypanosoma cruzi* (Y strain), maintained respectively in Schneider's and LIT medium. Parasites ($5x10^6$ cells/ml) were incubated in triplicate in 96-well microplates at 26^oC with different concentrations (0.8 a 1,000μg/ml) of the extracts and compounds. After 72h incubation, the activity was determined by counting the number of live parasites in Neubauer chambers. All experiments were repeated 3 times. As controls Amphotericin B (1μM), Benznidazole (50μg/ml) and DMSO 1% were used. Citotoxicity of active compounds was evaluated in Vero cells ($2x10^4$ cells/well) by the MTT method. Four of the tested compounds (crude extracts of *L. microcladia* and *S. chilensis*, Solidagenone and Elatol) were active against both *Leishmania* species and *T. cruzi*. The values of IC_{50} ranged between 7.32 to 9.92μg/ml for *L. amazonensis*, 3.76 to 13.43μg/ml for *L. chagasi* and 34.14 to 52.19μg/ml for *T. cruzi*. None of the active compounds showed toxicity to Vero cells in concentration below 100μg/ml. The low cell toxicity and the expressive activity of Elatol and Solidagenone against *Leishmania promastigotes* reinforces the need to investigate their activity against intracellular amastigotes. Both Elatol and Solidagenone may be promising prototype molecules for developing new antiparasitic compounds. Supported by CNPq.

QT17 - *In vitro* evaluation of leishmanicidal and trypanocidal activity of crude extracts of plants from the Brazilian Cerrado.

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The chemotherapy arsenal available for treatment of Chagas' disease and Leishmaniasis is restricted to few drugs which presented limited efficacy and show undesirable side effects. The Brazilian plant biodiversity represents a rich source for screening of new potential antiparasitic compounds. In present study the leishmanicidal and trypanocidal activities of twenty extracts obtained from Annonaceae, Sophoraceae, Sapotaceae and Apocynaceae families, from the Mato Grosso state flora was evaluated. Extracts were solubilized in DMSO (50mg/ml) and tested against promastigotes of *Leishmania amazonensis* (575 strain), *L. chagasi* (PP75 strain) and epimastigotes of *Trypanosoma cruzi* (Y strain) ($5x10^6$ cells/ml), maintained respectively in Schneider's and LIT medium. Parasites were incubated in triplicate in 96-well microplates at 26^oC with different concentrations (0.8 to 1,000μg/ml) of each extract. After 72h of incubation, the activity was determined by counting the number of live parasites in Neubauer chambers. Amphotericin B (1μM), Benznidazol (50μg/ml) and DMSO 1% were used as controls. Extracts active against epimastigotes were incubated with blood trypomastigotes at 4^oC and survival parasites determined after 48h. Citotoxicity of active extracts was evaluated using Vero cells ($2x10^4$ cells/well) by the MTT method. Four of the

tested extracts (*Xylophia aromatica*; *Bowdichia virgiloides*; *Aspidosperma cuspa*; *Acosmium dasycarpum*) reduced *L. amazonensis* growth (IC_{50} 4.5-61.05μg/ml). Two of them (*X. aromatica*, *A. cuspa*) were also active against *L. chagasi*, with IC_{50} values between 6.13 and 11.07μg/ml. Three tested extracts (*X. aromatica*, *A. cuspa* and "Ipê Pimenta') showed trypanocidal activity for epimastigotes (IC_{50} 44.35-115.73mg/ml) and hexanic extract of *A. cuspa* was active against blood trypomastigotes (IC_{50} 124.42 to 182.34μg/ml). Cell toxicity CC_{50} of the active extracts varied from 84.77 to 1,128.54μg/ml. These results show a promising antiparasitic activity in some crude extracts of Brazilian Cerrado plants. Studies with purified fractions and isolated compounds are under way for determination of the active compounds. Supported by CNPq.

QT18 - Antileishmanial Activity of *Stipopodium zonale* (SZE) is partially due to Atomaric acid

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Leishmaniasis, a disease that affects 12 million people worldwide, is found in five continents and is endemic in the tropical and sub-tropical regions. Recently, these numbers are increasing due to the co-infection with HIV. Pentavalent antimonials, still the first choice treatment for this infection, present several side effects and parasite resistance is been reported. All these stimulate the search for new anti-leishmanial agents. We have shown that a dichloromethane extract from the brown algae *Stypopodium zonale SZE* presented antileishmanial effect against promastigotes and amastigotes of *Leishmania amazonensis*. In this study we researched the antileishmanial activity of atomaric acid *ATOM* isolated from SZE. Our results shown here that ATOM, differently from SZE, presents leishmanicidal activity against *L. amazonensis* amastigotes but not for promastigotes. The leishmanicidal activity of ATOM was dose dependent, varying from 24 to 53% inhibition of amastigote growth, respectively with 0.1 and 10 nM ATOM. The amastigotes growth inhibition is independent of nitric oxide *NO* production, since ATOM treated macrophages were unable to modulate the NO produced by stimulated macrophages treated or not with LPS plus IFN-g. In order to test the safety of this compound for host cells, macrophages were treated with ATOM and cell viability was assessed using XTT and Trypan Blue dye exclusion assays. The results showed that ATOM at concentration of 10 nM is not toxic for macrophages, while at the concentration of 100 nM, a 30% toxicity was observed. These results suggest that ATOM is partially responsible for the leishmanicidal activity of SZE, but others compounds present in the SZE should be tested. Supported by: Capes, CNPq and Faperj.

QT19 - Macroalgae produce active molecules against *Leishmania amazonensis*.

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Sea macroalgae produce active substances against several diseases, but few works evaluating the antileishmanial activity have been accomplished. The aim of this study was evaluate the antileishmanial activity of different marine macroalgae crude extracts of the Brazilian coast. Different macroalgae species belonging to the Phaeophyta, Rhodophyta and Chlorophyta divisions were collected along the Brazilian coast from Pernambuco to Paraná states. A total of 54 species were submitted to different extractions for the preparation of organic and aqueous extracts. *Leishmania amazonensis* promastigotes were cultivated with different extracts in several concentrations in 199 medium supplemented with 10% fetal bovine serum, for 72h. Of the 65 evaluated extracts, 24 presented IC50 lower than 100ug/ml. Among these, five species had prominence: two red algae, *Acanthophora spicifera* with IC50 at 20,11ug/ml ± 1,75 and *Laurencia obtuse* with IC50 at 12,79ug/ml ± 1,23; two brown algae, *Dictyota menstrualis* with IC50 at 3,63ug/ml ± 0,77 and *Stypopodium zonale* with IC50 at 3,07ug/ml ± 0,7; and, the green alga *Caulerpa racemosa* with IC50 at 19,7ug/ml ± 1,43. These results indicate the potential of algae as natural resources to the discovery and development of new antileishmanial drugs. CNPq

QT20 - Effect of Epitaondiol, a secondary metabolite isolated from the Brown Algae *Stypopodium Zonale*, on the growth and morphology of *Trypanosoma cruzi*.

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Chagas' disease is an endemic disease in some areas of Latin America. About 18 million persons are infected with the etiological agent, *Trypanosoma cruzi*, and more than 100 million are living at risk of infection. The chemotherapy of the disease is still insufficient and ineffective in its acute and chronic stages. Many efforts have been made in recent years to know more about possible new biochemical targets to design new selective drugs. Marines seaweeds have potential cytotoxic activity against some diseases as cancer and AIDS, but little is known about the potential use against tropical diseases. The seaweed *Stypopodium zonale* which is very abundant at Brazilian Litoral, produces terpenoids that show

a broad pharmacological activity. Our purpose was to study the activity of the metabolite epitaondiol isolated fron the *S. zonale* from Atol das Rocas, Brazil, as a potential agent for Chagas disease treatment. We first used a culture of epimastigote forms of Y strain *T. cruzi* grown in LIT medium at 28 °C with different concentrations of epitaondiol for 24, 48, 72 and 96 hours. The IC50 value obtained for 96 hours was 8.00 μg
mL. Observations by light microscopy and scanning electron microscopy showed alterations in cell morphology. Transmission electron microscopy of treated epimastigotes showed mitochondrion swelling, presence of autophagosomes, loss of matrix eletron density and disorganization of the nuclear chromatin. On the other hand, *T. cruzi* infected macrophages treated with 3, 5, 8 and 10 μg
mL of epitaondiol for 96 hours showed little effect against the intracelular amastigote forms. Morphological changes in macrophages were observed only at concentrations above 8.00 μg
mL.

QT21 - Marine Invertebrate Metabolites as Antiprotozoan: *In vitro* activity of *Macrorhynchia philippina* against *Leishmania* (L.) *chagasi*.

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Protozoan parasitic diseases affect the poorest populations in the world, and consequently are not seen as potential markets. Visceral Leishmaniasis (VL) is a fatal and progressive disease caused by *Leishmania* (L.) *chagasi* in Brazil. The clinical therapy is limited by severe toxic effects, with pentavalent antimonials such as sodium stibogluconate and meglumine antimoniate as first line drugs. Amphotericin B deoxycholate is highly active but has extensive toxicity complications. The search for more effective and less toxic drugs is essential, and natural products may offer an unlimited source of chemical diversity for new drug templates. Especially for infectious diseases, the exploration of the marine environment represents a new and promising tool in the search of new active compounds. In order to find new effective compounds against *Leishmania* sp., we decided to study the *in vitro* activity of *Macrorhynchia philippina* methanolic crude extract against *L.* (L.) *chagasi*, and also its mammalian citotoxicity. The crude methanolic extract killed 100% of promastigotes with an Effective Concentration 50% (EC50) of 15.37 microg/mL and a Selectivity Index of 5.5-fold in mammalian cells. Through Adsorption Chromatography in silica columns, High Performance Liquid Chromatography fractioning and biomonitored assays, we have found very active fractions against promastigotes. Our findings indicate that this marine cnidarian has a promising antileishmanial activity and further isolation of active compounds could indicate novel and potential drug candidates. This work was supported by FAPESP.

QT22 - Effective Antiprotozoan Activity of a Polyketide Isolated From the Sponge *Plakortis angulospiculatus*

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Introduction. Marine invertebrate metabolites have provided promising drug prototypes for clinical therapy of many infectious diseases. In particular sponge metabolites have shown significant antiprotozoan, antiviral and antibacterial activities. A peroxide-containing metabolite, plakortide F, isolated from the sponge *Plakortis* sp., displayed significant activity against *Plasmodium falciparum*. Considering that Visceral Leishmaniasis and Chagas Disease are typical tropical pathologies in developing countries, and that only highly toxic, and in some cases, ineffective drugs are available for the treatment of such diseases, there is a necessity of the discovery of novel drug prototypes for the treatment of Leishmaniasis and Chagas disease. **Objectives**. In this work we have investigated the antileishmanial and antitrypanosomal activity of a metabolite isolated from the marine sponge *Plakortis angulospiculatus*. **Results**. The polyketide isolated from *P. angulospiculatus* displayed significant antileishmanial and antitrypanosomal activity, with low cytotoxicity against mammalian cells and no hemolytic activity. The polyketide was tested against *L. chagasi* promastigotes and intracellular amastigotes, with a 50% Effective Concentration of 1.91 microg/mL and 0.52 microg/mL, respectively. LLC-MK2-derived tripomastigotes of *Trypanosoma cruzi* (Y strain) were also susceptible to the polyketide, with a 50% Effective Concentration of 2.33 microg/mL, as verified by the MTT assay. The Selectivity Index (cytotoxicity against mammalian cells/activity against parasites) of the polyketide demonstrated excellent values for *L. chagasi* (32-fold) and for T. cruzi (7 fold). No hemolytic activity was observed up to 6 microg/mL using mouse erythrocytes. **Conclusions**. These results indicate that the polyketide isolated from the sponge *P. angulospiculatus* may be a useful drug prototype for drug design studies especially for neglected diseases caused by protozoan parasites. This work was supported by FAPESP.

QT23 - EFFECT OF *Crotalus viridis viridis* VENOM ON *Trypanosoma cruzi*

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Snake venoms are natural biological resource of compounds with therapeutic value, and have been used in treatment of pathophysiological conditions in medicine. Chagas disease chemotherapy is based on drugs that exhibit toxic effects and limited efficacy such as Nifurtimox and Benznidazole. Therefore, new chemotherapeutic agents from natural sources are a lining research to be exploited. This study shows that *Crotralus viridis viridis* venom affects *T. cruzi* epimastigotes and trypomastigotes, and do not present toxicity to $LLC-MK_2$ cells at concentrations that kill these parasites. Epimastigotes were cultivated at 28 ^{o}C for 4 days in LIT medium and tissue culture trypomastigotes for 24h at 37 ^{o}C in the presence of 0.2 to 500 μg/mL of *C.v.viridis* venom. The effect on parasites growth and lyses was evaluated by counting in a Neubauer chamber, and the morphology was verified by electron microscopy. To analyze the venom toxicity on a vertebrate cell, $LLC-MK_2$ cells were seeded in 24-well plates containing glass coverslips and cultivated in RPMI supplemented wit 10 % FCS containing 0.3, 0.6 and 1mg/mL of C.v.viridis venom for 5 days. Coverslips were collected daily and fixed in Bouin solution, stained with Giemsa to quantify the cells and analyse the effects on their morphology. The ED50 for epimastigotes inhibition growth after 1 day was 0.6 μg/mL. Trypomastigote lysis was observed in the presence of 0.3 μg/mL. The analysis at the electron microscope showed swelling of mitochondria and plasma membrane, changes on the aspect of cytoplamic compartments and changes of the parasite shape. Incubation of $LLC-MK_2$ cells with 0.3 and 0.6 mg/mL did not cause alteration on cell number and morphology. Our data showed that *C.v.viridis* venom was effective against *T. cruzi* at concentrations 10 times lower than those capable to affect the $LLC-MK_2$ cells.

QT24 - *In vitro* Activity of *Siphonops annulatus* Cutaneous Secretion against *Leishmania* (L.) *chagasi*

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Leishmania spp cause a spectrum of diseases, ranging from a cutaneous ulceration to a progressive and fatal visceral disease. The disease is transmitted by a sandfly vector to humans, dogs and some vertebrates. Leishmaniasis affects more than 12 million people worldwide with an increasing number of cases each year, especially in developing countries. Cutaneous secretions of amphibians have been demonstrating a valuable tool for Drug Discovery studies. The secretions consist of a myriad of compounds, as peptides, alkaloids, steroids and other organic compounds, which are produced as chemical weaponry against the predators. The secreted substances have shown potential activity against bacteria, fungi and parasites. In order to search organic compounds with antileishmanial activity in the crude cutaneous secretion, we have initially tested the methanolic extract against parasites and further developed a liquid-liquid partition of this extract using three different solvents: n-hexane, ethyl acetate and n-butanol. Test compounds were *in vitro* incubated with *Leishmania* (L.) *chagasi* promastigotes at 300 microg/mL, and after 24 h incubation, all four organic extracts presented a killing activity, as determined by MTT assay at 570 nm. The methanol extract presented of killing activity; n-hexane extract killed ; ethyl acetate killed and n-butanol extract killed of promastigotes. These promising data of *Siphonops annulatus* organic extracts suggest novel potential antileishmanial compounds, and chromatographic fractionation are under evaluation. This work was supported by FAPESP (05/00974-9).

QT25 - Antiparasitic activity of furamidine analogue (DB569) associated to benznidazole against Trypanosoma cruzi in vitro

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Trypanosoma cruzi is a pathogen with complex life cycle displaying distinct morphological stages. Currently, the drugs available for treatment of Chagas disease are nifurtimox and benznidazole (Bz) but, both compounds aren't effective against the chronic phase and exhibit considerable side effects, justifying the search for new therapies or their use in combination with other chemotherapeutic agents used at clinical in other pathologies. Diphenylfuran diamidines represent an important promising class of DNA-targeted antiparasitic agents. The best-known member of then is the DB75 commonly referred to as furamidine and related unfused aromatic diamidines that have been proven useful for the treatment of parasitic infections. We previously reported that one of its analogues, DB569, exhibits higher trypanocidal dose and time-dependent effects against different forms of T. cruzi as compared to DB75. Recent literature clearly points to the need of finding more efficient and less toxic chemotherapeutic approaches for Chagas disease, and that DB569 exhibits promissory protective effect against T. cruzi in vitro and in vivo. Our present aim was investigated its in vitro effects associated to Bz. Our results corroborated with previous reported that showed that both compounds have anti-parasitic effect against T. cruzi, ranging IC50 values of 0,66 and 10ìM after 24hs treatment for DB569 and Bz, respectively. When the treatment with Bz was in association with 2ìM of DB569, after 2hs treatment, the trypanocidal

effects was more potent (IC50 18,6ìM) than Bz alone (IC50 200ìM). In contrast, the treatment with DB569 (IC50 3ìM) didn't higher with 25ìM Bz addition (IC50 2,7ìM), after 2hs, but after 24hs of treatment the trypanocidal effects was increased about 10-fild (0,66 to 0,05, respectively). This significantly enhances of the anti-parasitic activity of Bz in association with DB569 appears as a promising candidate for new schedules of therapies, possibility reduce the dose of benznidazole.

QT26 - ANTILEISHMANIAL ACTIVITY OF SYNTHETIC BENZYL-NITROIMIDAZOLES DERIVED FROM MEGAZOL.

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Megazol is a nitroimidazole-derived drug with good *in vitro* and *in vivo* activities against *Trypanosoma brucei* in rodents and primate. However, these effects are also accompanied by high toxicity and mutagenicity in animals. Some megazol analogues were synthesized as an attempt to minimize its toxicity, whereas preserving its therapeutical effect against *T. brucei* and *T. cruzi*. Here, we evaluated the activity of 4 trypanosome-active analogues (43A, 44A, 45A, and 60A) on *Leishmania amazonensis*. Promastigotes de *L. amazonensis* transfected with Green Fluorescence Protein (GFP) were cultivated for 72h in the presence of several concentrations of the test substances in 199 medium supplemented with 10% of fetal bovine serum. We found that Megazol and the analogue 60A were the most active against *L. amazonensis*, with IC50 of 4,88±1,88 uM and 8,84±2,02 uM, respectively. The IC50 of analogues 43A, 44A and 45A was 13,16±1,50 uM, 11,21±0,96 uM and 30,56±0,65 uM, respectively. These results show that in addition to their anti- *T. brucei* and anti-anti- *T.cruzi* activity, both Megazol and compound 60A are also active against promastigotes of anti-*L. amazonensis*. The activity against intracellular amastigotes and toxicity to mammal cells is in progress to demonstrate compound 60A as an ammeliorated version of Megazol and its potential as a new nitroimidazole antileishmanial drug.

QT27 - A quick assay to evaluate benznidazole susceptibility of *Trypanosoma cruzi* isolates. Analysis of isolates from patients submitted to chemotherapy

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INTRODUCTION Two drugs are available for treatment of *T. cruzi* infection: nifurtimox and benznidazole (BZ). National policy in several countries is to offer treatment to patients under 15 years of age. This policy could be extended to adults. By using experimental models it has been shown that *T. cruzi* isolates present different susceptibilities to the two drugs. This aspect may be responsible for differences in the efficacies of chemotherapy. GOAL This study aimed at developing a quick assay to evaluate BZ susceptibility. RESULTS - Epimastigotes were incubated in LIT medium in 24-well plates in the absence or presence of seven BZ concentrations. The IC50 values were determined after parasite counting. For eleven strains the IC50 varied from 7 to 32 ±M. BZ susceptibility of six of these strains was previously assessed in experimentally infected mice (Filardi & Brener, 1987). For these strains we observed a correlation between the IC50 values and the classification of these strains in susceptible or resistant. BZ susceptibility was also determined in tissue culture-derived trypomastigotes (TCT). Epimastigote controls were run in parallel. For a given strain, IC50 for TCT was lower (30-40%) than that of epimastigotes. Nevertheless, the relative degree of susceptibility was maintained. The assay was also performed with isolates from chronic patients of Minas Gerais submitted to BZ treatment. The IC50 of three isolates of individuals with positive hemoculture before treatment and repeatedly negative several years after treatment were: 19.2±4; 19.4±4 and 35±4. The IC50 of isolates of four patients before and after BZ treatment (therapeutic failure) was also determined: 16 μM (two patients); 26 μM (one patient); 50 μM (one patient). The IC50 of the isolates before and after treatment did not change. CONCLUSION - We found no correlation between the IC50 value of a given isolate and the therapeutic success or failure.

QT28 - Antiprotozoal activity *in vitro* of some synthetic chalcones

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Nowadays, treatment of Leishmaniasis and Chagas disease present severe side effects and limited efficacy, emphasizing the importance to search new compounds against these diseases. Chalcones have emerged as an important class with antiprotozoal potential, especially against *Leishmania* spp. In the present study we evaluated the leishmanicidal and trypanocidal effects of eleven modified chalcones (CL1 to CL11), changing the substituents on rings A and B, according to Topliss method. Promastigotes of *Leishmania amazonensis*

(575 strain), *L. chagasi* (PP75 strain) or epimastigotes of *Trypanosoma cruzi* (Y strain) ($5x10^6$ cells/ml) of Schneider and LIT medium, respectively, were incubated in 96 well plates at 26°C with serial dilutions of the compounds. After 72h incubation, the activity was determined by hemocytometer counting. As control were used amphotericin B (1μM), benznidazol (50μM) and DMSO 1%. Citotoxicity of active compounds was evaluated in Vero cells ($2x10^4$ cells/well) by the MTT method. Although neither of substituted chalcones showed trypanocidal effect, leishmanicidal activity was significantly increased, mainly against *L. chagasi*.The values of IC_{50} against *L. amazonensis* ranged 13.5 to 273.4μM while against *L. chagasi* the IC_{50} was $<$ 10 to 146.2μM. Among the eleven tested chalcones, six of them showed strong effect against promastigotes of *L. chagasi* (IC_{90}= 18.8 to 79.8μM). Except for CL11, which present one bromo atom on the ring A, all the chalcones exerted citotoxicity lower than its activity against *L. chagasi*. Antiparasitic assays of these chalcones against *Leishmania* intracellular amastigotes are under investigation. Our results showed promising leishmanicidal activity in some of these substituted chalcones. The studies on application of Topliss method are now in progress. Supported by ProBIC/ProPPEC/UNIVALI and CNPq.

QT29 - Synthesis and *in vitro* Evaluation of Leishmanicidal and Trypanocidal activity of N-quinolin-8-yl-arylsulfonamides

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Currently, the drugs used for treatment of Leishmaniasis and Chagas disease are highly toxic and present limited efficacy. Thus, the development of new therapeutic compounds is urgently required. In the present study we evaluated the leishmanicidal and trypanocidal effect of eleven synthesized N-quinolin-8-yl-arylsulfonamides against both extra and intracellular forms. Epimastigotes of *Trypanosoma cruzi* (Y strain), promastigotes of *Leishmania amazonensis* (575 strain) and *L. chagasi* (PP75 strain) were incubated in a 96 well plates during 72 hours with serial dilutions of the compounds in LIT 10% FCS and Schneider 5% FCS, respectively. The results were analyzed by hemocytometer counting. Citotoxicity was evaluated against VERO cells using MTT method. The ability for reduction of intracellular amastigote infection was evaluated using Vero cells and peritoneal murine macrophages infected with *T. cruzi* and *L. amazonensis*, respectively. Nine of the eleven compounds tested showed leishmanicidal activity against promastigotes. Compound 6, which contains a pyridine moiety, was the most active against *L. chagasi* (IC_{50}= 0.56μM) and 10, the quinolinesulfonamide derivative, the one with best potential against *L. amazonensis* (IC_{50}= 2.12μM). Only compounds 6 and 17 showed *T. cruzi* epimastigotes toxicity, wich IC_{50}= 31.75 and IC_{50}= 4.10μM, respectively. When tested against *L. amazonensis* infected murine macrophages and *T. cruzi* infected Vero cells, compound 6 at 25μM demonstrated a reduction of cell infection of 84.8% and 96.4% respectively. In addition, the copper complex 17 showed a high leishmanicidal and trypanocidal activity (IC_{50}= 1.10 to 4.10 μM) against both extra and intracellular forms. In conclusion, this work indicates that the N-quinolin-8-ylarylesulfonamides possesses significant antileishmanial and trypanocidal activity against both intra and extracellular forms. There are also indications that specific structural changes in the quinoline moiety drastically affect the antiprotozoal activity. Supported by CNPq and CAPES

QT30 - *In vitro* trypanocidal and leishmanicidal activity of sulfonamide 4-methoxychalcone derivatives

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Leishmaniasis and Chagas disease continues to cause significant morbidity and mortality, mainly in the developing world. Currently available chemotherapy for these diseases, are unsatisfactory due their limited efficacy and undesirable side effects. Thus, ten sulfonamide 4-methoxychalcone derivatives were synthesized and evaluated *in vitro* against *Trypanosoma cruzi* (Y strain) and *Leishmania amazonensis* (575 strain). Hundred-eighty microlitters of *T. cruzi* epimastigotes (10^7/ml) and *L. amazonensis* promastigotes (10^7/ml) were incubated in triplicate in 96 well plates with 20 μl of different compounds concentrations (100 to 1.6 μM) at 26°C for 72 hours in LIT 10% FCS and Schneider 5% FCS, respectively. Parasite survival was evaluated by counting of live flagellates in a hemocytometer. Citotoxicity was evaluated against J774.A1 cell line in vitro using the MTT method. For intracellular leishmanicidal activity evaluation J774.A1 cell line was incubated with Leishmania cultured amastigotes in a parasite cell rate of 5:1 overnight at 34°C under gentle shaking. Cells were seeded on circular 13mm glass slides and cultivated for 48 hours at 34°C 5% CO_2 in the presence of 25μM, 5μM and 1μM of each compound. After that, slides were fixed with methanol, giemsa stained and mounted in microscope glass slides. Two hundred randomly chosen cells were counted and the infection rate evaluated. Nine out of ten compounds showed a concentration-dependent growth inhibition of *Leishmania* promastigotes (IC_{50} 4.3-43.7μM), with no significant toxic effect towards J774.A1 cell line. Three compounds (CR54, CR57, CR50)

reduced the infection cell rate ranging from 84% to 54% at 25μM, as well as decreased the number amastigotes. None of the test compound showed trypanocidal activity. Our results show that the combination of two different pharmacophoric groups, chalcone and sulfonamide, enhance the leishmanicidal activity of the compounds. Supported by Cnpq and Capes.

QT31 - A new application to selective estrogen receptor modulators: Tamoxifen and Raloxifene as novel antileishmanial candidates

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Tamoxifen -TAM- and Raloxifene -RAL- are selective estrogen receptor modulators, SERMs, widely used in the treatment of breast cancer. We previously demonstrated that TAM is effective against *Leishmania* parasites in vitro acting independently of the estrogen machinery. In this work we show the antileishmanial activity of RAL, a compound also approved for prevention of osteoporosis. RAL inhibited the viability of *Leishmania amazonensis* amastigotes with IC50 % of 15 +/ - 2.3 microM) and *L. amazonensis*, *L. braziliensis*, *L. chagasi*, *L. donovani*, *L. major* and *L. mexicana* promastigotes in vitro with IC50 % values ranging from 30 to 40 microM, approximately. Differently from TAM, which exhibits time-dependent antileishmanial activity, RAL presented practically the same efficacy after 2, 24 and 48 h of incubation with *L. amazonensis* promastigotes. This discrepancy can be attributed to structural divergences in these molecules, given that TAM is a triphenylethylene derivative and RAL contains a benzotiophene ring, which could suggest the existence of specific mechanisms of action for each drug. Since RAL has been reported to be a potent inducer of nitric oxide -NO- synthase in endothelial cells, the production of NO after treatment of infected macrophages with RAL was investigated. Infection rates on resident peritoneal macrophages infected in vitro decreased by 40.0 and 77.6 % when cells were treated with 25 and 30 microM RAL, respectively. However, preliminary results indicated that the drug did not exacerbate NO production in infected macrophages. Taking RAL's efficacy against *Leishmania* into consideration and bearing in mind that this drug has a well established clinical use we suggest that RAL has a great potential to be tested as a therapeutic agent against leishmaniasis. Supported by FAPESP.

QT32 - *In vitro* inhibitory effect of Tamoxifen on the proliferation of *Trypanosoma cruzi*

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Chagas disease remains the largest parasitic disease burden in Latin America, despite recent advances in the control of the transmission in some parts of the continent. This important parasitosis caused by the protozoan *Trypanosoma cruzi* affects nearly 16 million of people. Specific treatment based on the nitroderivates, Benznidazole (BZ) and Nifurtimox (NX) is unsatisfactory, due to limited efficacy, particularly in the prevalent chronic stage, and frequent deleterious side effects. There is considerable interest in the development of new drugs for the treatment of Chagas disease. The Tamoxifen (TAM) is an antiestrogen drug used in the treatment of the breast cancer. The objective of this work was to evaluate the *in vitro* effect of TAM on the proliferation of *T. cruzi*. Epimastigote form of the BZS (benznidazole-susceptible) and BZR (benznidazole-resistant) *T. cruzi* strains were cultivated in Liver infusion tryptose medium, supplemented with new born calf serum at 28^{o}C. Cultures were initiated at a density of 2x10^6/ml epimastigotes and drugs (TAM or BZ) were added in different concentrations (800μM, 400μM, 200μM, 100μM, 50μM, 25μM and 12,5μM). After seven days of incubation, was determined the IC_50 and IC_90 values (50% and 90% growth inhibitory concentration, respectively) for each drug. TAM had a strong effect, inhibiting completely the parasite multiplication between concentrations of 50 and 800μM. The drug inhibited parasite multiplication at low concentrations, with IC_50 of 12μM (BZS strain) and 16μM (BZR strain). The IC_90 for both strains was of 45μM/ml. BZ presented lower activity than TAM against *T. cruzi*. IC_50 value for BZ was 26μM (BZS strain) and 45μM (BZR strain). The IC_90 was of 90μM (BZS strain) and 180μM (BZR strain). Our results show that TAM is a potent *in vitro* inhibitor of *T. cruzi*, and suggested that drug merit *in vivo* studies. Supported by: IRR/FIOCRUZ

QT33 - Naphthoquinoidal [1,2,3]-triazole, a new structural moiety active against *Trypanosoma cruzi*

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Although the recent advances in the control of the transmission of Chagas disease, we are still challenged by two critical problems: the treatment of chronic cases of the disease and the high level of acute cases in Latin America. In this context, an intensive research program has been focused upon the search for alternative natural drugs. Our group is involved in the synthesis and evaluation of the try-

panocidal activity of lapachol and beta-lapachone and derivatives being these naphthoquinones extracted from the heartwood of *Tabebuia* trees (Bignoniaceae) (reviewed in Pinto et al., 2007). Continuing our studies, we will investigate compounds with naphthofuranquinoidal endowed linked to a triazolic moiety. [1,2,3]-Triazoles are an important class of heterocycle due to their wide range of biological activities such as antiplatelet agents (Cunha et al. 2003), anticonvulsants (Kelley et al. 1995) and antimicrobial agents (Costa et al. 2006;). The triazolic nucleus and the naphthoquinone ring are moieties with independent biological activities and in this context the Huisgen cyclization under copper catalysis was employed. Nor-lapachol (1) was obtained from lapachol by Hooker oxidation, and bromo-â-nor-lapachone (2) was prepared through cyclization of 1 with bromine in chloroform. From 2, through nucleophylic substitution with sodium azide in dichloromethane was obtained the azide 3, the key intermediate for the synthesis of quinones coupled to the triazolic nucleus, employing 1,3-dipolar reaction between the azidoquinone and an alkyne, catalyzed by Cu(I), known as "click chemistry". We synthesized and characterized five derivatives of 1, and assayed their activity against trypomastigotes. All the derivatives were more active than the original quinones, with IC50/1 day values in the range of 17 to 359 microM, being the apolar phenyl substituted triazole 6 the most active compound. These triazole derivatives of nor-beta-lapachone emerge as interesting new lead compounds in drug development for Chagas disease.

QT34 - Therapeutic activity of a new synthetic naphthoquinone in the experimental murine leishmaniasis.

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Quinones have been largely studied for antitumoral, molluscicidal, antiparasitic, anti-inflammatory, antifungal, antimicrobial, and trypanocidal activities. In our previous studies, the synthetic naphthoquinone derivatives LQB 17 and 118 presented strong in vitro activity on both forms of Leishmania, without significant toxicity to macrophages until 20 uM, and did not affect the nitric oxide production by infected macrophages until 40 uM. These results demonstrated for the first time the selective antileishmanial activity of these compounds and indicated a direct action on the parasites, once they did not modulate the NO production by macrophages. In the present work, we evaluated the therapeutic activity of these compounds in the experimental murine leishmaniasis model. BALB/C mice were infected with $2x10^6$ promastigotes of Leishmania amazonensis on the footpad. The experimental groups were treated twice a week with 100uM of the compounds and the control ones with PBS or 200ug of Pentostam by intralesional injections. The lesion growth was accompanied with a dial caliper. Animals were sacrificed after 25 doses (105 days after the infection), the parasitic load evaluated by LDA and the serum was collected for creatinine, ALT and AST analysis. The compound LQB 118 was able to inhibit the lesion growth as efficiently as Pentostam and did not induce significant alterations in the evaluated parameters of renal and hepatic toxicity. LQB 17 was not able to inhibit the lesion growth in this treatment regimen. Altogether, these results indicate that the structural differences between LQB 17 and 118 can be decisive to the bioavailability of these compounds and LQB 118 can be a potential lead compound for developing new leishmanicidal drugs.

QT35 - Effects of naphatoquinones on Leishmania (Viannia) braziliensis cysteine- and serine- proteinases activities.

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The search for more specific chemotherapies to treat leishmaniasis with less adverse side effects is still an relevant topic nowadays. Naphtoquinones from the heartwood of *Bignoniaceae* and *Verbanaceae* trees are known by their significant anti-microbial properties and, also to induce the formation of reactive oxygen species and inhibit topoisomerases activities. Still today, naphtoquinones have not been included in drug screenings for leishmaniasis. In previous studies naphtoquinones derivates showed good activity against *T.cruzi* (Ferreira et al. 2006; Jorqueira et al, 2006) and *L.(V) braziliensis*. The aim of this study is to aid in the knowledge of possible chemotherapy drugs, evaluating the inhibitory activity of three naphtoquinones derivatives (α-Lapachone,β-Lapachone and Oxyran 10) over the activities of *Leishmania(Viannia) braziliensis* promastigotes proteinases. The chromogenic substrates used to assess enzymes activities were: pGlu-Phe-Leu p-nitroanilide (for cysteine-proteinase), in 10 mM sodium acetate buffer pH 5.5 containing 1 mM dithiothreitol (for cysteine-proteinase) and Nα-p-Tosil-l-Arg methyl ester hydrochloride in 10 mM Tris-HCl pH 7.5 (for serine-proteinase). The drugs β-Lapachone, α-Lapachone and Oxyran 10 were dissolved (50 μM) in DMSO and commercial inhibitors, as E-64 and PMSF (50 μM), were used to confirm the specificity of proteinases activities. The enzymatic assays performed with specific chromogenic substrates revealed that β-Lapachone inhibited mainly cysteine-proteinase activity (6.65 x 10^{-3} μmoles minute^{-1} mg of

protein^{-1}), while Oxyran 10 inhibited serine-proteinase (1.6 x 10^{-4} μmoles minute^{-1} mg of protein^{-1}) in promastigotes protein extracts when compared to total activities for both enzyme classes (1 x 10^{-2} μmoles minute^{-1} mg of protein^{-1} and 3.03 x 10^{-4} μmoles minute^{-1} mg of protein^{-1}, respectively). Other proteinases activities are yet to be assayed, allowing us then to define the inhibition profile of *L. (V.) braziliensis* proteinases by naphtoquinones. This is important for further studies to the development of new drugs to control the leishmaniasis, based on lapachone analogs.

QT37 - ANTILEISHMANIAL ACTIVITIES OF PURINE DERIVATIVES

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The drugs of choice for the treatment of leishmaniasis are pentavalent antimonials, but toxic side effects, limited efficacy to control parasite proliferation and drug resistance are frequently encountered. Considering it, the search for more effective drugs against leishmaniasis became necessary. In order to find new drugs against leishmaniasis we decided to check the *in vitro* effect of 6-(α-aceticacidthio)purine, 6-(3‘-(thioethylamine)propylthio)purine and salt of fluor of the 8,9-dihydro-7H-[1,4]thiazepino[3,2,4-hi]purine. These substances were assayed against *Leishmania amazonensis* amastigotes because these derivatives presented activity against *Leishmania amazonensis* promastigotes. The concentrations of substances were 39 and 78 μM for 6-(α-aceticacidthio)purine, 50 and 100 μM for 6-(3‘-(thioethylamine)propylthio)purine, 28 and 56 μM for salt of fluor of the 8,9-dihydro-7H-[1,4]thiazepino[3,2,4-hi]purine using cells J774A.1 culture infected with 1,67x10^7 parasites/ml. The results expressed as the number of parasite by macrophage after the period of treatment with substances. A few biological activity was noticed for amastigotes in 6-(3‘-(thioethylamine)propylthio)purine of the used concentrations. But the treatment infected amastigotes J774A.1 cells cultures with 6-(α-aceticacidthio)purine and salt of fluor of the 8,9-dihydro-7H-[1,4]thiazepino[3,2,4-hi]purine led to a decrease of the amastigotes proliferation. These reduction for 6-(α-aceticacidthio)purine were 49.4% in 24 hours of treatment, 28.7% in 48 hours and 42.6% in 72 hours for the concentration 78 μM and 37.5% in 24 hours, 50.5% in 48 hours and 44.6% in 72 hours for the concentration 39 μM. The reduction for salt of fluor of the 8,9-dihydro-7H-[1,4]thiazepino[3,2,4-hi]purine were 36.8% in 24 hours of treatment and 18.2% in 48 hours for the concentration 56 μM and 44.1% in 24 hours and 22.4% in 48 hours for the concentration 28 μM. No biological activity was noticed for amastigotes in salt of fluor of the 8,9-dihydro-7H-[1,4]thiazepino[3,2,4-hi]purine of the used concentrations after 72 hours of treatment. None of the substances were found to have significant toxicity effect on macrophages. Supported by FAPEMIG, UFJF.

QT38 - LEISHMANICIDAL ACTIVITY OF N-ALKYL ETHYLENEDIAMINE DERIVATIVES

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Chemoterapeutic treatment of leishmaniasis usually relies on the use of pentavalent antimonials, but toxic side effects, limited efficacy to control parasite proliferation and drug resistance are frequently encountered. The second-line compounds used during the treatment of unresponsive cases generally include pentamidine and amphotericin B. There is an urgent need for safer and more efficacious anti-Leishmania agents. Our purpose is to investigate the effect of N-alkyl ethylenediamine derivatives against promastigotes of *Leishmania amazonensis* and *L. chagasi*. The substances tested were: *N*-octyl-ethylenediamine, *N*-decyl-ethylenediamine, *N*-dodecyl-ethylenediamine, *N*-tetradecyl-ethylenediamine and *N*-hexadecyl-ethylenediamine. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The result expressed as the concentrations inhibiting parasite growth by 50 % (IC_{50}) after three days incubation period. All substances assayed, *N*-octyl-ethylenediamine, *N*-decyl-ethylenediamine, *N*-dodecyl-ethylenediamine, *N*-tetradecyl-ethylenediamine and *N*-hexadecyl-ethylenediamine, inhibited the growth of promastigote forms of *L. amazonensis* (IC_{50} values of 7.2 v, 3.9 μM, 0.94 μM, 4.88 μM and 13.2 μM, respectively) and *L. chagasi* (IC_{50} values of 2.1 μM, 3.4 μM, 0.26 μM, 4.99 μM and 3.2 μM). These results point new perspectives by the leishmaniasis treatment. Further experiments are being carried out in order to investigate cytotoxicity against mammalian cells, besides analysis with amastigote forms for a better study of this new approach for the chemotherapy of leishmaniasis. Supported by CAPES, CNPQ and UFJF.

QT39 - THE ANTI-LEISHMANIAL EFFECT OF N- ALKYL AMINO ALCOHOLS

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The clinical manifestations of leishmaniasis may range from single cutaneous lesions to fatal visceral leishmaniasis. Conventional chemotherapy relies on multiple parenteral injections with pentavalent antimonials that are considerably toxic and prone to induce resistance. Second-line drugs, such

as Amphotericin B and its lipid formulations, are either too toxic or expensive for routine use in developing countries. These facts call for safer, cheaper, and more efective new antileishmanial drugs. The aim of this study is to define the antileishmanial activity of *N*-alkyl amino alcohols against promastigotes of *Leishmania amazonensis* and *L. chagasi*. The substances tested were *N*-octyl-ethanolamine, *N*-decyl-ethanolamine, *N*-dodecyl-ethanolamine and *N*-tetradecyl-ethanolamine. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The results expressed as the concentrations inhibiting parasite growth by 50 percent (IC_{50}) after three days incubation period. Among the four substances tested, only one, *N*-tetradecyl-ethanolamine, not showed activity against *L. amazonensis* and *L. chagasi* promastigotes. The others substances, *N*-octyl-ethanolamine, *N*-decyl-ethanolamine and *N*-dodecyl-ethanolamine showed an activity against promastigote forms of *L. amazonensis* (IC_{50} values of 76.4 μM, 11.7 μM and 8.57 μM, respectively) and *L. chagasi* (IC_{50} values of 9.65 μM, 5.5 μM and 5.42 μM, respectively). Some derivatives showed activity against promastigote forms of *Leishmania* showing therapeutic potencial to the treatment of the leishmaniasis. Further tests will be made in order to determine the cytotoxicity effect against mammalian cells and analysis with amastigotes of *Leishmania*. Supported by CAPES, CNPQ and UFJF.

QT40 - Activity of some N-alkyl-1,3-propanediamine derivatives against promastigotes of *Leishmania amazonensis* and *L. chagasi*

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The development of new leishmanicidal agents is extremely important, considering the high toxicity of the clinical drugs and in some cases a none completely efficiency. Its treatment with antimonials, amphotericin-B or pentamidine is associated with multiple side effects making the search for new treatments imperative, because the long course of treatment allows high levels of the drug to accumulate in the tissues, namely the liver and spleen. In order to find new drugs against leishmaniasis we decided to check *in vitro* the effect of *N*-octyl-1,3-propanediamine, *N*-decil-1,3-propanediamine, *N*-tetradecyl-1,3-propanediamine and *N*-hexadecyl-1,3-propanediamine. These substances were *N*-alkyl-1,3-propanediamine derivatives. All compounds were assayed against *L. amazonensis* and *L. chagasi* promastigote forms. Each concentration was screened in triplicate and it was performed in flat-bottomed 96-well plastic tissue-culture plates. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The results expressed as the concentrations inhibiting parasite growth by 50 percent (IC_{50}) after three days incubation period. All substances assayed inhibited the growth of promastigote forms of *Leishmania*. *N*-octyl-1,3-propanediamine, *N*-decil-1,3-propanediamine, *N*-tetradecyl-1,3-propanediamine and *N*-hexadecyl-1,3-propanediamine, respectively, they had a significant activity against promastigotes of *L. amazonensis* (IC_{50} of 7.2 μM, 7.2 μM, 3.1 μM and 76.9 μM) and *L. chagasi* (IC50 of 2.1 μM, 0.73 μM, 2.70 μM and 83.6 μM). These data show that these substances exhibit clearly a activity against *L. amazonensis* and *L. chagasi* suggest therapeutic potential these compounds to the treatment. So we will make experiments about citotoxicity against mammalians cells and we will assay against intracellular amastigotes of *Leishmania* for a better study about activity of these drugs. Suported by UFJF, CAPES and CNPQ.

QT41 - Activity of reversed diamidines against *Trypanosoma cruzi in vitro* and *in vivo*

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Aromatic diamidines present broad-spectrum activity towards different pathogens. However, although possessing high anti-parasitic activity in vitro and in vivo, these compounds present high toxicity and lack oral biodisponibility limiting their clinical use. To overcome these restrictions, several analogs and prodrugs have been developed, including the reversed amidines, promissory compounds that display an excellent activity in vitro against fungi, bacteria and protozoan such as Trypanosoma cruzi, the eatiological agent of Chagas' disease. Then, due to the poor efficacy and considerable side effects of the available drugs used for treating Chagas' disease, our aim was to investigate the tripanocidal activity of DB766, a reversed amidine (RA), against T. cruzi (Y strain) through in vitro as well as in vivo studies. Our data showed that DB766 displays an excellent trypanocidal effect towards bloodstream trypomastigotes (BT) incubated at 37^{o}C for 24h, displaying an IC50 value of 0.05μM. Aiming the possible use of this compounds also in the profilaxis of banked blood, we next evaluated the effected of DB766 against BT incubated at 4^{o}C for 24h in the presence of whole mice blood. Our results showed that in the presence of blood, this RA kept a very good trypanocidal activity, reaching an IC50 value of 0.16μM. Moreover, the analysis of drug toxicity performed by incubating uninfected Vero cell with crescent doses of DB766 showed that only 9% of the cultures displayed loss of cellular viability when the higher dose (96μM) was assayed. Then due the promissory selective index (LD50/IC50) of this compound (>1900x) in vitro, our next step was evaluate the effect of DB766 during the infection of male Swiss mice with 104 parasites of T. cruzi (Y). The preliminary

results showed a marked decrease in the parasitemia levels when the infected mice were treated with two doses (via i.p) of 50mg/Kg DB766.

QT42 - Leishmanicidal Evaluation of the Amidine and Imidazole Pyrazoles Derivatives

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Leishmaniasis is a disfiguring and sometimes fatal disease caused by protozoan parasites of the genus *Leishmania*. Chemotherapy for leishmaniasis is currently inadequate. Therefore, the search for novel, effective and safe therapeutic compounds has become a priority. In this study, we analyzed the anti-Leishmania activity of the novel synthetic compounds including four 4-amidine-1-aryl-1H-pyrazoles and twelve 4-amidine-1-aryl-4- (4,5-dihidro-1H-imidazole-2-yl)pyrazoles. The in vitro cytotoxic effects of these derivatives on the host cells were also determined. These compounds were assayed against promastigote forms of Leishmania amazonensis (MHOM/BR/77/LTB0016 strain). Parasites were cultured with and without the drugs in Schneider's medium at 26°C, using Pentamidine Isethionate as reference drug. After 24 hours incubation, the anti-Leishmania activity was determined by addition the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) and could be read in spectrophotometer with wavelength of 490 nm. This procedure is also used to observe the toxic effects of these compounds in peritoneal macrophage. The preliminary results showed that the class of the imidazole pyrazoles were more effective against promastigote forms of *L.amazonensis* than amidine pyrazoles derivatives. Furthermore, all these derivatives presented lower toxicity in murine macrophages than the reference drug. These results provide perspectives for the development of new imidazoles derivatives due it is interesting to point out that imidazole rings are present in several known antiparasitic drugs.

QT43 - Functional analysis of two loci of Leishmania (L.) major related to resistance with Tubercidin, a toxic adenosine analog

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Design of selective antiparasite drugs depends on the exploitation of fundamental biochemical differences between parasites and host. In leishmania, an important metabolic difference is based on the incapacity of de novo purine synthesis making this parasite dependent of exogenous purines to growth. Starting from an overexpression/selection method, we isolated and mapped two different L. (L.) major loci related with resistance to the toxic adenosine analog, Tubercidin (TUB). One locus contains a previous described toxic nucleoside resistance (the TOR gene). The role of TOR in the purine mechanism seems to be related to redirect adenosine permeases from the plasma membrane to the vesicular tubule lysosome. Cells became TUB resistant because they were unable to take up and accumulate this toxic purine. The second and different locus is capable to confer two-fold TUB resistance for wild type lines after transfection/overexpression, and according the GenBank data base, codifies for a hypothetic protein located at chromosome 31 of L. major. Functional analyses with the over expressed lines of L. major transfected with both loci are carrying out with and without the presence of specific inhibitors of TUB transporters. We expect that a better understand of the mechanism of purines biosynthesis in leishmania, can contribute for new therapeutic insights for leishmaniasis.

QT44 - Identification and chromosomal localization of 9 different Leishmania (L.) major loci related with resistance to inhibitors of the ergosterol biosynthesis

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Leishmaniasis causes significant morbidity and mortality around the world. It is endemic in 88 countries, with approximately 400.000 new cases/year. The first line drug for chemotherapy is based on pentavalent antimony that requires parenteral administration and high-dose courses to achieve a clinical response, rendering toxicity and drug resistance. Ergosterol is an essential component responsible to maintain the integrity and fluidity of leishmania membranes. Starting from an overexpression/selection method, we isolated and mapped nine different L. (L.) major loci related with resistance against two inhibitors of ergosterol biosynthesis, Terbinafine (TBF) and Itraconazole (ITZ). Individual functional analysis of these nine loci in the presence of TBF and/or ITZ present low, but significant, levels of resistance (ranging from 1,5 to 2,5 fold resistance) after transfection and overexpression in wild type lines. Most of them present cross-resistance with both inhibitors; and two cosmids render cells resistance to TBF and hypersensitivity to ITZ after transfection, suggesting that more than one locus can be related with the Ergosterol biosynthesis in the same insert. Besides one of the locus encodes the TBF target Squalen Monoxigenase, in silico analysis based on the leishmania genome data base indicates that none of the others loci codifies proteins directly related to the ergosterol biosynthesis. Chromosome and southern blot analysis confirm that the nine loci are dif-

ferent and not related. Together, these findings suggest that leishmania seems to be capable to develop different mechanisms to survive with TBF or ITZ treatments, what indicated that inhibitors of the Ergosterol biosynthesis must be considered not to be used as single agent in leishmaniasis chemotherapy.

QT45 - Effect of Tomatidine on *Phytomonas serpens*

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Some plants produce substances for their own defense against pathogens and predators. In *lycopersicon* species, such as tomato *L.lycopersicon*, the main antimicrobial compound is the steroidal glycoalkaloid α-tomatine. The loss of tetrasaccharide side chain of tomatine forms the aglycone tomatidine. Flagellates of the genus *Phytomonas* are etiologic agents of diseases affecting plants of great economical importance, including coffee, coconut, tomato and many others. In the present study we describe tomatine and tomatidine as inhibitors of grown of *Phytomonas serpens* and its effects on lipid composition and morphological cell changes. When tomatine was added in cells culture, almost 100% of cell death was observed in the first 24 hours of contact with the drug. On the other hand, tomatidine did not kill the cells, but stopped cellular proliferation and caused morphological changes, like decrease of cellular length, vacuolization and shortening of flagellum. By thin-layer chromatography, an evident reduction in the ergosterol level after tomatidine treatment was observed. Besides, through fluorescence microscopy was shown that cells treated with tomatidine strongly accumulated the probe Bodipy-Fatty acid in vacuoles. The results indicate that tomatine and tomatidine have different molecular target.

QT46 - Treatment with the iron quelator desferrioxamine in association with benznidazole on the evolution of the experimental infection by *Trypanosoma cruzi*

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The nutritional status of the host can influence the host-parasite relationship. Iron ions play an important role in *Trypanosoma cruzi* infection. In this study we evaluated the effects of iron deprivation in association with benznidazole on several aspects of the infection of Swiss mice by the Y strain of *T. cruzi*. Thirty days old male mice were kept in stainless steel cages and fed a non-purified diet. Animals were divided into six groups: the infected not-treated group (INT) which received sterile water, a group infected and treated which received intraperitoneal injections of desferrioxamine (DFO1) for 21 days starting at the first day of parasitemia, a third group infected and treated with benznidazole (BZ) for 21 days starting at the first day of parasitemia, a group infected and treated with DFO in association with BZ (DFO+BZ), a group treated with DFO 14 days prior to infection and continuing up to 21 d.p.i (DFO2) and a lest group treated with association of (DFO2+ DFO+BZ). Fourteen days after treatment (DFO2) and (DFO2+ DFO+BZ) were inoculated with 500 blood stage forms of *T. cruzi*. Animals were sacrificed in 10th e 16th d.p.i. Iron serum levels of infected animals were decreased in INT and increased in group DFO2+DFO+BZ when compared to INT. Hemoglobin levels were also decreased in infected animals from group (DFO2+DFO+BZ). Infected animals from the DFO2 group showed a increased spleen and liver size when compared to the other groups. Parasitemia and associated mortality were higher in animals from the INT group while animals from the (DFO2+ DFO+BZ) group showed 0% of mortality. Thus, it appears that the alterations observed in the parasitemia and mortality may not be related to alterations in the host immune system but rather be associated with the availability of iron for the parasite. Supported by CNPq, FAPEMIG, UFOP

QT47 - TECHNOLOGICAL DEVELOPMENT OF PHB-HV MICROPARTICLE CONTAINING AN ANTILEISHMANIAL CHALCONE FOR THE TREATMENT OF LEISHMANIASIS

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New polymeric microparticulate systems have been used for controlled drug delivery to ameliorate drug pharmacokinetics and reduce toxicity. Biodegradable biopolymers such as microbial polyhydroxyalkanoates (PHAs) have been industrially produced as substitutes for non-degradable plastics. Poly(D-3 hydroxybutyrate) (PHB) produced by a variety of bacteria as energy and carbon storage material is the most studied PHA for pharmaceutical purposes, due to its high

biodegradability and biocompatibility. In this work, we evaluated the feasibility of the use of poly(hydroxybutyrate-co-hydroxyvalerate) P(HBHV) microspheres to effectively deliver an antileishmanial drug, the nitrogenated chalcone CH8 developed by our group. The P(HBHV) microparticles were prepared by an emulsion/solvent evaporation process by dissolving the copolymer and CH8 (10:1) in chloroform, using polyvinyl alcohol (PVA) as surfactant. The formed particles were then washed, filtered and dried. To estimate the rate of drug encapsulation, the HPLC-DAD method was used to generate a calibration curve for CH8 quantitative analysis ($r^2 = 0.9953 + -0.0026$, $n = 3$). A RP-18 reverse-phase column and 100% acetonitrile as mobile phase was used in an isocratic system. Flow elution was 1 mL $min^{-}1$, 20 uL samples were injected and spectra were recorded in 377nm. Triplicate HPLC determinations were performed on each sample. The drug content of the microspheres was measured using dichloromethane to extract the encapsulated chalcone. The microparticle analysis used the same conditions reported for drug analysis. We found that the CH8 content in the particles was 9.91%, indicating a high (98 %) rate of incorporation. The P(HBHV)-CH8 microparticles will serve for the evaluation of improved efficacy of the chalcone CH8 both *in vitro* and *in vivo* in mice infected with cutaneous and visceral leishmaniasis. Financial support: Capes.

QT48 - Ultrastructural and biochemical effects of miltefosine in *Crithidia deanei*, an endosymbiont-bearing trypanosomatid

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Crithidia deanei is a Trypanosomatidae that presents an obligatory intracellular bacterium, which maintains a mutualistic relationship with the host. The endosymbiont envelope lacks sterols and the phospholipid composition is characterized by a major quantity of cardiolipin (CL), followed by phosphatidylcholine (PC) and phosphatidylethanolamine (PE). In prokaryotes, PC is present only in species closely associated with eukaryotes, either in symbiotic or pathogenic interactions. In this study, we tested the effects of miltefosine in *C. deanei*, a drug that inhibits the CTP: phosphocholine citidyltransferase, a key enzyme in the PC biosynthesis of eukaryotic cells. The miltefosine presented low effect on protozoa cell proliferation after 36h of treatment with 10, 25 and 50μM of the drug. However, when a higher concentration (100μM) was used, the cell proliferation was strongly inhibited. Assays with aposymbiotic cells of *C. deanei* were also performed in order to verify if the endosymbiont influences the phospholipid biosynthesis in *C.deanei*. Data obtained by transmission electron microscopy, showed that miltefosine promoted ultrastructural effects in *C. deanei*. Thus, after 24 hours of treatment with 25μM miltefosine, cells showed plasma membrane shedding and blebbing, the endosymbiont envelope showed convolutions and the mitochondrion presented swelling. Biochemical analyses will be performed to check the phospholipid composition of drug treated protozoa and symbionts obtained after cellular fractioning. Insight about the mechanisms underlying phospholipid biosynthesis in *C.deanei* might help to understand how the prokaryote/trypanosomatid relation has evolved in the establishment of symbiosis. Supported by: CNPq, FAPERJ

QT49 - Effect of the HIV protease inhibitors on promastigote forms of *Leishmania amazonensis*

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After the beginning of the highly active antiretroviral therapy (HAART) in the treatment of the AIDS, the incidence, morbidity and mortality for the viral, bacterial, fungal and parasitic co-infections, diminished drastically. There is evidence that HIV protease inhibitors have a direct inhibitory effect on the parasites. The present study was undertaken to analyze the effect of nelfinavir and indinavir, two HIV aspartic protease inhibitors, on promastigote growth of *Leishmania amazonensis*. The promastigotes were collected from late log phase of growth and incubated in microplates with the compounds at final concentrations ranging from 10 to 400 μM and the rate of multiplication was assessed by counting the parasites using a haemocytometer chamber. Our results showed that nelfinavir and indinavir have a dose-dependent antileishmanial activity in vitro. These results suggest that HIV aspartic protease inhibitors could be an interesting alternative chemotherapy in the treatment of *Leishmania*-HIV co-infection. Supported by: MCT/CNPq, CEPG/UFRJ, FAPERJ and FIOCRUZ.

QT50 - Effect of a Dolabellane Diterpene in the *Leishmania* Cell Cycle and Leishmanicidal Activity in HIV-1 Co-Infected Human Macrophages

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Leishmania are protozoan that cause an ample spectrum of diseases. There are no effective vaccines, and chemotherapy is expensive and produces several side effects. Besides,

drug resistance constitutes an increasing problem, mainly in the immunocompromised patients, such as HIV-infected. In these cases an exacerbated growth of *Leishmania* occurs in different tissues. All these facts stimulate the search for new anti-leishmanial agents. Dolabellane diterpenes present antimalarial and anti-HIV-1 inhibitory activities. We have shown that 8,10,18-trihydroxy-2,6-dolabelladiene (Rocatriol), obtained from the brown algae *Dictyota pfaffii*, possessed leishmanicidal activity against intracellular amastigotes of *Leishmania amazonensis* (IC50 44,5 uM). This activity is not due to modulation of NO levels, an important mechanism for *Leishmania* killing, since Rocatriol-treated macrophages produced the same amount of nitrite as untreated macrophages. Actually, Rocatriol was able to reduce NO production induced by LPS plus INF-g-activated-macrophages. Here, we further studied cytokine modulation in Rocatrioltreated macrophages. Ours studies showed that the leishmanicidal effect of Rocatriol is independent of IL10, TNFα and TGF-β production. Thus, we investigated the Rocatriol effect on the promastigote cell cycle. Promastigotes treated with 50 uM of Rocatriol for 24 and 48h were stained with propidium iodide and analyzed by flow cytometry. We found that Rocatriol induced a 75% decrease in the number of cells in G2 phase of the cell cycle, suggesting a possible direct activity in the promastigotes. Finally, we tested Rocatriol activity in Leishmania-HIV-1 co-infected human macrophages, which mimic the exacerbated growth of both pathogens observed in patients. It was found that 50uM of Rocatriol inhibited 50% of the amastigote growth in HIV-1 co-infected macrophages. The results presented here suggest a direct effect of Rocatriol in the parasite cell cycle, and that this compound could be active against the protozoan even when its growth is exacerbated by a co-pathogen. Supported by: CAPES, CNPq and Faperj.

QT51 - Leishmanicidal activity induced by novel Hydrazones associated to 24-Sterol Methyltransferase blockade

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Leishmaniasis a not controlled emergent disease around the world (category I/ WHO, 2005), demands sustained searching for new effective chemotherapeutic alternative. 24-Sterol Methyltransferase (24-SMT) only present in lower eukaryotes constitutes an excellent differential cell target to be evaluated in the present work. In this sense 20-hydrazone-imidazoline-2-yl-5α-pregnan-3β-ol (Hydra1) and 22-hydrazone-imidazoline-2-yl-5-colen-3β-ol (Hydra2) at concentrations between 0.5 and 10 μM were assayed against *Leishmania (L.) mexicana* (NR strain) and *Leishmania (V.) braziliensis* (M2903 strain) promastigotes for 48 h, respectively. Parasites were maintained at exponential phase of growth in Schneider's insect medium supplemented with 10% foetal bovine serum at 26 °C. Cell density was followed by direct counting in Neubauer chamber and viability by trypan blue exclusion. Sterols analysis were carried out by gas chromatography and mass spectrometry whereas ultrastructural study by TEM employing conventional methods. Additionally, Hydra2 citotoxicity on human lymphocytes was determined by fluorescence microscopy. Hydrazones induced an antiproliferative dose dependent effect on both species of *Leishmania* with estimated MIC for Hydra1 and Hidra2 of 9.5 μM and 3.7 μM, respectively. Mainly sterols 5-dehydroepisterol and episterol present in *L.(L.) mexicana* and 5-dehydroepisterol and ergosterol present in *L.(V.) braziliensis* disappeared with concomitant accumulation of the same sterols colesta-5,7,24-trien-3β-ol and colesta-7,24-dien-3β-ol when parasites were treated with corresponding IC_{20} of Hydrazones. Consistently with sterols composition drastic change ultrastructural study using IC_{50} of both Hydrazones showed alterations principally related to biomembranes, characterized by mitochondrial integrity loss and plasmatic membrane evaginations. Preliminary assays carried out with MIC of Hydra 2 not induced citotoxicity on human lymphocytes. These findings suggest that potent leishmanicidal activity induced by novel Hydrazones could be associated to 24-SMT blockade constituting a promising differential cell target not present in vertebrates to be evaluate in experimental model of Leishmaniasis *in vivo*. Supported by Fonacit G-2005000827

QT52 - Na^+-ATPase and PKC: the potential targets of miltefosine against *Trypanosoma cruzi* growth

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Miltefosine is the new oral drug used in the treatment of visceral leishmaniasis in humans. It has also been demonstrated its activity against the causative agent of Chagas disease, *Trypanosoma cruzi*. In general, the effects of miltefosine are associated to changes in phospholipids metabolism. The observation of miltefosine inhibiting the phospholipase C-β, PKC and Na^+,K^+-ATPase activities in mammalian cells suggests that this drug has a spreading action in the *T. cruzi* including ion transporters and cell signaling. In a recent study, an ouabain-insensitive Na^+-ATPase was cloned and showed to be encoded by the *TcENA* gene and expressed in plasma

membrane. Moreover, epimastigotes overproducing *TcENA* showed increased tolerance to high Na^+ stress. Therefore, we decided to evaluate the role of Na^+-ATPase and PKC activities as potential target to miltefosine in *T. cruzi*. To verify the action of miltefosine on the activity of the Na^+-ATPase and PKC in *T. cruzi*, epimastigotes culture of y-strain was grown and the membrane fraction (MF) was used. The Na^+-ATPase activity in MF was measured using [γ-^{32}P]ATP as substrate. The PKC activity was measured through the phosphorylation of histone H1. Our studies demonstrated that furosemide, a specific inhibitor of Na^+-ATPase, inhibited in 80% the *T. cruzi* growth similar to the effect obtained with 0.5μg/ml miltefosine. In addition, miltefosine inhibited the Na^+-ATPase activity in a dose-dependent and reversible manner. This inhibition was completely reversed when the enzyme was solubilized with 0.1% deoxycholate indicating the involvement of interaction between the enzyme and membrane phospholipids on the drug effect. It was also observed that miltefosine inhibited the PKC activity but these effects are not interdependent. Altogether, our results indicate that miltefosine inhibits *T. cruzi* growth through, at least in part, the inhibition of both Na^+-ATPase and PKC activities. Supported by: CAPES, CNPQ, FAPERJ.

QT53 - CHANGES IN LIPID METABOLISM BY A NATURAL CHALCONE AND ITS NITRO-SUBSTITUTED SYNTHETIC ANALOGUE

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Previously, we demonstrated the strong in vitro and in vivo activities on *Leishmania amazonensis* of the natural chalcone DMC (2‘,6‘-dihydroxy-4‘-methoxychalcone) and its synthetic nitro-substituted analogue (CH8). To further analyze the mechanism of action of those chalcones, we looked at their effects on the lipid profile of treated parasites. *L. amazonensis* promastigotes were cultured in the presence of 15 μM DMC or 7.5 μM CH8 in medium 199 plus 10% fetal bovine serum for 72 h. After extensive washing in saline, the cell numbers were adjusted. The lipids were extracted with chloroform/methanol (2:1), dried with N^2 and then submitted to thin-layer chromatography in hexane:diethilether:acetic acid (60:40:1) followed by hexane:chloroform:acetic acid (80:20:1), and visualized with Charring reagent. The thin-layer chromatography (TLC) bands were then analyzed by densitometry. We found that both chalcones DMC and CH8 interfered significantly with the parasite lipid metabolism. Decreased triglycerides (35% and 53%), ergosterol (34% and 46%) and monoglycerides (39% and 48%) was observed for DMC and CH8-treated parasites, respectively. For phospholipid analysis, promastigotes were incubated with the chalcones as above, for 24 h in the presence of 20uCi/ml of inorganic ^{3}2P. Phospholipids were extracted with chloroform/methanol (2:1), dried with N^2 and then submitted to TLC in chloroform:acetone:methanol:acetic acid:water (40:15:13:12:8). Commercial phospholipids were included as standards. The autoradiography and densitometry analysis showed a decrease in phosphatidic acid (67% and 65%), phosphatidyletanolamine (65% and 17%), phosphatidylcoline (63% and 15%) and phosphatidylglycerol (33% and 10%) in DMC and CH8-treated parasites, respectively. On the other hand, increased sphingomyelin (13 and 183%) and lysophosphatidylcoline (11% and 30%) content was found. These results show that both chalcones DMC and CH8 interfere not only with the parasite sterol biosynthesis as shown previously, but also with other neutral lipids and phospholipid metabolism.
CAPES and CNPq.

QT54 - Fatty acid biosynthesis inhibitors thiolactomycins as potential anti-*Toxoplasma gondii* agents

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The discovery of new chemotherapies against *Toxoplasma gondii* is extremely important due to the severe disease caused by this pathogen in immunocompromised hosts and to congenital infection. The plastid-like organelle, the apicoplast, performs several important metabolic functions which are “plant-like”in origin. These often have significant differences to the corresponding mammalian pathways and hence there are a number of potential drug-targets in apicoplast metabolism. One of these pathways is the type II fatty acid synthesis (FAS II). In this work we studied the inhibitory effect of 8 thiolactomycin (TLM) analogues, known inhibitors of prokaryotic β-ketoacyl-acyl carrier protein synthase in FAS II, against RH strain tachyzoite-infected LLCMK2 cells. The TLM analogues demonstrated potent anti-*T. gondii* activity, arresting tachyzoite proliferation with IC50 values in the micromolar level, ranging from 1.6μM up to 40μM after 48h of treatment. The rapid reduction of parasite load suggested that these compounds have selective cytotoxic effects against *T. gondii*. Transmission electron microscopy demonstrated that TLM analogues interfered with membrane-bounded organelles. The main effects observed were in Golgi complex which showed swollen cisternae and intense vacuolization after 48h of treatment, and a clear inability of the tachyzoites to complete the division, since large poly-nucleated parasites resembling cells in schizogony were visualized, and this in turn affected parasite development and survival. This work was supported by CNPq, FAPERJ and Cardiff Partnership Fund for funding (IHG, SMJ).

QT55 - Antileishmanial activity of new hydroxyethylpiperazines used as precursors in the synthesis of HIV protease inhibitors

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Recently, the antileishmanial activity of the HIV protease inhibitors has been demonstrated, possibly due to the parasite proteasome inhibition. In this work, we aim to evaluate the antileishmanial activity of new hydroxyethylpiperazines used as precursors in the synthesis of the HIV protease inhibitors, saquinavir and lopinavir. *Leishmania amazonensis* promastigotes were cultivated with eight test compounds (PIMCs), saquinavir and lopinavir. After 96 h, the parasite growth was evaluated by MTT. The PMIC 02, 03, 04, 05 and 08 were active, with IC50 lower than 50 μM on promastigotes. Lopinavir exhibited a strong antipromastigote activity, with IC50 lower than 15 μM, while the IC50 of saquinavir was 35 μM. The active compounds were then evaluated on intracellular amastigotes. Murine peritoneal macrophages were infected with *L. amazonensis* and treated with test compounds. After 72 h, the cells were stained and the intracellular amastigotes were counted. PIMC 02, 05 and 08 were able to inhibit the parasite growth more than 90 % at 50 μM, while PIMC 04 inhibited 86 % at the same concentration. The antiamastigote activity of PIMC 03 and lopinavir at 50 μM was not determined, once these compounds were completely toxic to macrophages in this concentration. So, to evaluate the citotoxicity of all tested compounds, supernatant from the cultures was analyzed in relation to lactate dehydrogenase (LDH) content, a cytoplasmic enzyme. Maximum LDH liberation was obtained with PIMC 03 and lopinavir at 50 μM. The LDH liberation of PIMC 02, 05 and 08 treated cells ranged from 25 % to 70 %, while no specific LDH liberation was observed in PIMC 04 treated macrophages. Altogether, these results indicate that PIMC 04, a methoxylated hydroxyethylpiperazine, could be a potential prototype to development of new antileishmanial drugs, once it was able to inhibit significantly the amastigote growth, without apparent toxicity to macrophages. CNPq

QT56 - Study of leishmanicidal activity of quinolyl-N-alkyl phosphoramidates of diisopropyl derivatives

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Leishmaniasis are caused by protozoan parasites of genus *Leishmania*, that can occur in different clinical manifestations such as cutaneous, mucocutaneous and visceral, and constitutes a serious public health problem. As drugs currently clinic used are toxic and mostly inefficient, searching for new leishmanicidal drugs is necessary. In this study the *in vitro* effect of anti-*Leishmania (L) amazonensis* (strain MHOM/BR/77/LTB0016) of five derivatives of 7-NO2 or 8-Y-quinolyl-N-alkyl phosphoramidates of diisopropyl (where Y is H, Cl or CH3) against promastigote forms and the most active among the derivatives was assayed against intracellular amastigostes. The promastigotes were incubated with and without the drug in Schneiders medium at 26^{o}C, using Pentamidine as reference drug. After 96 hours, the leishmanicidal activity was evaluated through the addition of MTT (tetrazolium bromide) and analyzed in 490 nm using a spectrophotometer. The intracellular amastigotes were obtained by infection of *L. amazonensis* in peritoneal murine macrophages and the activity of the derivative 8-Cl quinolyl was evaluated after 72 hours of incubation, when the cells were colored with Giemsa and counted in optic microscope. Preliminary results showed that among the tested compounds, the substitutes 8-Cl (IC50 = 93,3 $\pm$9,0μM) and 8-CH_3 (IC50 = 119,8 $\pm$ 15,0 μM) presented activity against promastigote forms. Furthermore, the amount of 50 μM of the derivative 8-Cl was able to reduce in 58 % the infection of macrophage. This result can be promising, due to the intracellular amastigote is the form responsible for the disease in mammalian. PIBIC-CNPq, PDTIS-FIOCRUZ

QT57 - *Trypanosoma cruzi* MITOCHONDRIAL DISRUPTION INDUCED BY C-ALLYL LAWSONE DERIVATIVES

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Naphthoquinones were broad distributed in plant kingdom. The abundance of natural sources of quinones in Brazil, encouraged us to develop together with group of Dr. Antônio Ventura Pinto, synthesis routes for quinones derivatives (Pinto et al., 2007). Naphthoquinones isolated from *Tabebuia* and their synthetic heterocyclic derivatives are the subject of our screening of new compounds with trypanocidal activity. Among more than 60 compounds synthesized up to now, we identified three naphthoimidazoles obtained from beta-lapachone as the most active compounds against blood-

stream trypomastigotes. New naphthoquinones were synthesized from C-allyl lawsone, their structures established and their activity against *T. cruzi* evaluated (Silva et al., 2006). These studies observed the trypanocidal activity of furanic napthoquinones. In continuity, we investigated the ultrastructural effects of C-allyl-lawsone derivatives against *T. cruzi* as well as biochemical and flow cytometry analysis were also performed. Ultrastructural analysis of *T. cruzi* epimastigotes treated for 24 h with the three naphthoquinones showed the mitochondrial swelling, the formation of blebs in the plasma membrane as well as the formation of concentric membranar structures inside organelles such as mitochondrion. Flow cytometry analysis confirmed the morphological data, suggesting that the mode of action of quinones 3222, 3223 and 3226 involves the disruption of mitochondrial membrane potential of parasites, as previously described for beta-lapachone derivatives (Menna-Barreto et al., 2005, 2007). All the quinones inhibited the activity of mitochondrial electron transport chain complexes I and III in both epimastigotes and trypomastigotes, whereas complex IV activity was not affected in these two parasite forms. Further investigations will be performed to characterize the specific mechanism of action of these compounds. The trypanocidal activity of these naphthoquinones stimulates extended studies with new analogues with redox properties, reinforcing the strategy of a rational approach in of the development of drugs active against Chagas disease.

QT58 - Effects of a DNA intercalating agent on ultrastructure and mitochondrial metabolism of trypanosomatids

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The *Trypanosomatidae* family comprises flagellated potozoa which present a typical structure, the kinetoplast, which contains the mitochondrial DNA (kDNA) arranged in catenated circles. This unique organization of the kDNA network and the susceptibility of the kinetoplast to a great variety of compounds, make this structure a potential target to chemotherapy. The kDNA presents different arrangements that vary according to species and stage of development, being also modified by the presence of an endosymbiont. In this work we analysed the effects of acriflavine, a DNA intercalating agent, in trypanosomatid growth, on kinetoplast ultrastructure and mitochondrion activity. In order to analyze the effect of acriflavine on proliferation of epimastigotes of *Trypanosoma cruzi* and *Blastocrithidia culicis*, cells were cultivated with different drug concentrations. Part of the culture was removed for cell counting and fixed for transmission electron microscopy. Cytochemistry analyses were also performed in order to identify nucleic acids and basic proteins. Our results showed that acriflavine promoted a dose-dependent inhibitory effect on the cell proliferation of both species analyzed. Biochemical approaches also demonstrated the acriflavine ability to decrease the oxygen rate of consumption on *T. cruzi* and *B. culicis*, indicating that the energetic metabolism of mitochondrion was affected in protozoa treated with this drug. TEM analysis showed that the acriflavine treatment promoted drastic ultrastructural modification in the kinetoplast. Staining of thin sections with 0.42% uranyl acetate indicated sites containing nucleic acids in the kinetoplast. The PTA technique, which reveals proteins rich in histidines, showed an intense staining in the condensed kDNA, but not in electron lucent areas of the disk, which probably correspond to decatenated circles. Similar results were observed with the amoniacal silver method, which reveals proteins rich in arginine and lysine. Taken together, these suggest that histone-like proteins are involved in the kDNA rearrangement after acriflavine treatment.

QT59 - *Leishmania (Viannia) braziliensis* in vivo model for testing new topical chemotherapeutic agents

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Chemotherapy of leishmaniasis is mainly based on the use of pentavalent antimonials, toxic drugs administered by the parenteral route. *L.(V.) braziliensis* is the main species that cause cutaneous leishmaniasis in Brazil and South America, but this is notoriously the most difficult *Leishmania* species to cultivate in vitro or to use in experimental infections in the murine model. Therefore, the aim of this study was to establish a detailed model for in vivo infections with virulent *L.(V.) braziliensis* isolates for testing of topical antileishmanial drugs. *L.(V.) braziliensis* isolates were collected from patients in Goiás (WSS-05 and UAF-06) and Bahia (BA788) states. *L.(V.) braziliensis* promastigotes (1 x 10^5) in 10 μL of saline solution were inoculated into the left ear dermis of female BALB/c mice. Inocules of 5 x 10^5 parasites in 50 μL of saline solution were injected into the left footpad and tail. Mice were followed up for twelve weeks and the lesion sizes were measured with a caliper. The isolate WSS-05 induced ear lesions apparent at week 3, reaching maximum size (1.4 ± 0.6 mm) at week 10, and remaining unchanged in size up to week 12. Footpad lesions were also observed reaching 1.8 ± 0.14 mm at week 12. With UAF-06 and BA788 isolates, the ear lesion reached maximum size at week 10 (0.8 ± 0.5 mm) and week 5 (1 ± 0.4 mm), respectively and healed completely by week 12. Footpad swelling was observed after UAF-06 and BA788 infections, beginning at week 6 and lasting until week 12. No mice developed detectable tail lesions.

In this work, we show that female BALB/c mice infected on ear dermis with *L.(V.) braziliensis* isolates developed lesions. Detailed follow up of lesion development will be performed through histopathological analyses and quantification of the parasite burden. Supported: FAPESP and CNPq.

QT60 - The inhibitory effects of the calpain inhibitor MDL28170 against *Trypanosoma cruzi*

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Peptidases of microbial pathogens have attracted the attention of many laboratories because of their roles in pathogenesis. Analysis of proteolytic enzymes of pathogenic organisms might lead to the design of powerful chemotherapeutic agents. In this context, several calpain inhibitors are under development and some are useful against important human pathogens. In this study, we report the effect of MDL28170, a potent calpain inhibitor, on the growth of *Trypanosoma cruzi* clone Dm28c. Briefly, epimastigotes were counted using a Neubauer chamber and resuspended in fresh medium to a final concentration of 5.0 x 10^6 viable epimastigotes per milliliter. The inhibitor was added to the culture at final concentrations of 30, 50, 60 and 70 μM. The calpain inhibitor at 70 μM promoted a powerful reduction on the cellular growth rate by approximately 80% after 24 h and 90% in 48, 72 and 96 h. Based on the effects of MDL28170 on the growth rate of *Trypanosoma cruzi*, we aimed to detect calpain homologues in this protozoan by immunoblot assays using different anti-calpain antibodies. The antibody raised against cytoskeleton-associated protein of *Trypanosoma brucei* (CAP 5.5) strongly recognized a polypeptide band migrating at approximately 80 kDa. No common epitopes were found between *Drosophila melanogaster* calpain and *T. cruzi* polypeptides. These results add new in vitro insights into the exploitation of calpain inhibitors in treating parasitic infections and add this family of proteases to the list of potential targets for development of more potent and specific inhibitors against trypanosomatids. SUPPORTED BY: MCT/CNPq, CEPG/UFRJ, FAPERJ

QT61 - Evaluation of D-ribone lactone derivates against *Leishmania sp.*

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Organisms of *Leishmania* genus are responsible for a several diseases collectively called as leishmaniasis, that affects 12 million people around the world and comprise three clinical forms: visceral, cutaneous and mucocutaneous. The chemotherapy of leishmaniasis is still based on pentavalent antimonials diaminas and antifungal polyene, but these drugs are general toxic, expensive, prone to generate resistance and require long-term treatment, which complicate its conclusion. The present study evaluated the effect of D-ribone lactone derivates against two different species of *Leishmania*: *L. amazonensis* which has been associated with all clinical forms of leishmaniasis and *L. chagasi* which is the causal agent of visceral disease. The viability of promastigotes was checked using the tetrazolium-dye (MTT) colorimetric method. The result expressed as the concentrations inhibiting parasite growth by 50 percent (IC_{50}) after three days incubation period. The compounds tested were: *N*-[2-(decylamine)-ethyl]ribonamide; *N*-[2-dodecylamine)-ethyl]ribonamide; *N*-[3-(decylamine)-propyl]ribonamide; *N*-[3-(dodecilamine)-propyl]ribonamide. All of these exhibited greater inhibitory effects on promastigostes forms of *L. chagasi* presenting IC_{50} values of 2.3, 19, 18.2 and 4.3 μM, respectively. Among the four tested compounds, three, *N*-[2-(decylamine)-ethyl]ribonamide; *N*-[2-dodecylamine)-ethyl]ribonamide; *N*-[3-(dodecilamine)-propyl]ribonamide showed an activity against *L. amazonensis* (IC_{50} values of 35; 47.4 and 11.8 μM, respectively) promastigote forms. In the next stages, the citotocixity against mammalian forms will be evaluated. Supported by UFJF, CAPES and CNPQ.

QT62 - Screening of hidrazidic compounds with potential leishmanicidal activity

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Leishmaniasis comprises a group of several different parasitic diseases in humans, is distributed worldwide and remains a severe public health problem. The disease is caused by the obligatory intracellular protozoan parasites of the genus Leishmania that multiply as the amastigota form in the macrophages of their vertebrate hosts. Pentavalent antimonials in the form of meglumine antimoniate (Glucantime) or sodium stibogluconate (Pentostam) are the mainstay drugs for systemic treatment of cutaneous leishmaniasis. However, these drugs as well as other drugs, are expensive, may be associated with numerousisde effects and have the requirement for intramuscular or intravenous injection. An intensive effort is being made in search for more effective drugs for chemotherapy of leishmaniasis. The aim of the present work was to develop new synthetic compounds with antileishmanial activity. In order to obtain pentamidine derivatives, strategies including bioisosterism and molecular

simplification were used to give rise to four series of hidrazidic compounds. In an attempt, to evaluate the efficacy of the new compounds in inhibiting the growth of intracellular, amastigotas of Leishmania we have used elicited peritoneal macrophages from BALB/c mice infected with L. major (cepa LV39, MRHO/Sv/59/P) or L. amazonensis (cepa Josefa, MHOM/BR/75). The standard anti-leishmanial drug pentamidine was used as a control based on its leishmanicidal activity. We have used two approaches to quantify the number of parasites: count of motile promastigotes in culture supernatant and the number of intracellular forms in infected macrophages. Our preliminary results indicated that 6 out of 23 hidrazidic compounds were highly effective in controlling both L. major and L. amazonensis amastigote replication.

QT63 - EFFECTS OF AMIODARONE ON MORPHOLOGY AND ULTRASTRUCTURE OF *TRYPANOSOMA CRUZI.*

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Chagas' disease has no effective treatment for the prevalent chronic form. The antiarrhythmic compound amiodarone, frequently prescribed for the symptomatic treatment of Chagas' disease patients, has also been shown to have antifungal and anti-*Trypanosoma cruzi* activity. Previous work have reported that amiodarone has direct activity against *T.cruzi*, both *in vitro* and *in vivo*, disrupting the Ca^{2+} homeostasis of the parasites and blocking ergosterol biosyntesis. We now show here for the first time the effects of amiodarone on the ultrastructure and morphology of epimastigotes and intracellular amastigotes of *T. cruzi*. Amiodarone had a clear, dose-dependent effect on proliferation of the epimastigote (extracellular) stage, with an IC_{50} of 9.75 μM. Against the clinically relevant intracellular amastigote form of the parasite, the drug was more potent with an IC_{50} of 4 μM, indicating a selective effect of this drug against *T. cruzi in vitro*. Transmission electron microscopy analysis of treated intracellular amastigotes and epimastigotes showed mitochondrion swelling associated with an electron-lucent matrix, alterations in the plasma membrane, lipid bodies and cytoplasmic vacuolization. Scanning electron microscopy analysis of treated epimastigotes showed detachment of the flagella from the cell body, as well a rounding of parasite body. These results demonstrate that amiodarone affects the structural organization of *T. cruzi*.

This work was supported by CNPq, Pronex-Faperj.

QT64 - Activity of palladacycle complexes against *Leishmania (Leishmania) amazonensis*

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The recommended drugs used for the treatment of leishmaniasis are pentavalent antimonials which were introduced 60 years ago. The main problems concerning the use of these compounds are toxicity and resistance. Thus, the development of new leishmanicidal drugs is an important goal and several compounds have been shown to have variable efficacy. Biphosphinic palladacycle complexes with antitumor activity act on neoplasic cells by the lysosomal pathway. Given the mechanism of action of these compounds and the phagolysosomal nature of the parasitophorous vacuoles harboring amastigotes in *Leishmania*-infected macrophages, the present studies evaluated the leishmanicidal activity of two palladacycle complexes, DPPE11 and DPPE12. The two compounds were first tested on the growth of *Leishmania (Leishmania) amazonensis* promastigotes in axenic medium. These experiments showed that three days of incubation with 156 nM DPPE11 and DPPE12 completely blocked growth of *L. (L.) amazonensis* promastigotes. The effect of these compounds was then tested on *L. (L.) amazonensis* amastigotes by the treatment of infected mouse peritoneal macrophages. The compounds were added at 1,000 nM to 100 μM 24 h after macrophage infection by *L. (L.) amazonensis* amastigotes and the cultures were examined after 72 h. At micromolar concentrations both drugs were toxic to macrophage cultures as determined by the MTT assay. A significant, dose-dependent decrease in infection index was observed with both drugs, with inhibition of 82% for 1,000 nM DPPE12 and 90% for 350 nM DPPE11. Nearly of total inhibition on *L. (L.) amazonensis* infection was observed when the infected macrophages were treated for 120 h with both compounds. These results point to the potential use of these palladacycle complexes as leishmanicidal drugs. *In vivo* assays of these compounds on this and other species of *Leishmania*, particularly *L. (L.) chagasi*, are currently in progress.

Supported by FAPESP.

QT65 - ACTIVITY OF CARBOLINE N-BUTYL-1-(4-DIMETHYLAMINO)PHENYL-1,2,3,4-TETRAHYDRO-β-CARBOLINE-3-CARBOXAMIDE) AGAINST AMASTIGOTE FORM OF *Trypanosoma cruzi*

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Trypanosoma cruzi is the causative agent of Chagas disease, which afflicts 18 million people in Latin America. The available treatment is limited and new compounds with trypanocidal effect must be found. Carboline compounds showed biological activities like, anti-tumoral, insecticidal, and anti-parasitic. In this study was evaluated anti-trypanosomal activity of N-butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide (carboline C4), against free and intracellular amastigote forms of *T. cruzi*. Amastigotes derived from tissue culture were added in microplate with drug solution at final concentrations of 8 to 256 μM and incubated at 37 ^{o}C or 4 ^{o}C /24 h. The viability of the cells was determined with 0.4% erythrosine B and EC50 (concentration which lysis 50% of the parasites) was calculated. Crystal violet was used as reference. To evaluate the effect of the drug on intracellular form, LLCMK2 cells were infected with bloodstream trypomastigotes and treated with carboline C4 at final concentrations of 16 to 128 μM. The survival index (product of the percentage of cells infected and the number of amastigotes by cell) was determined. For scanning electron microscopy, parasites were fixed, dehydrated, critical point-dried, sputter-coated with gold, and observed in Shimadzu SS-550 SEM. Citotoxicity of the drug was evaluated on LLCMK2 by sulforhodamine B assay. Carboline C4 showed dose-dependent effect in free amastigote with EC50 of 33 μM at 37^{o}C and 149 μM at 4^{o}C. LLCMK2 cells infected with amastigote and treated with carboline C4 showed a dose-dependent trypanocidal activity with survival index 50% at 17 μM. Scanning electron microscopy showed reduction of the size, alterations in the shape and cell body. Carboline C4 presented CC50 of 462 μM. This compound should be considered as a promising agent for the treatment of Chagas‘ disease, however further assays in vivo are necessary.

QT66 - Studies Toward the Structural Optimization of New Brazilizone-Related Trypanocidal 1,3,4-Thiadiazole-2-Arylhydrazone Derivatives

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Megazol, is active compound against Trypanosoma cruzi, and its action was associated with interference with parasite oxygen metabolism, as well as its role as thiol scavenger for the trypanothione. Trying to circumvent this, analogues belonging to a new class of 1,3,4-thiadiazole-2-arylhydrazone derivatives have been assayed against T. cruzi. It was identified the new trypanocide agent, Brazilizone A, IC50=5.3uM. With this result, we explore structurally-related 1,3,4-thiadiazole-2-arylhydrazone derivatives, in order to get a better understanding of the relationships between its structure and the antiprotozoal activity. We report the trypanocidal profile of Brazilizone A analogues, which supported the construction of 3D-QSAR models. The derivatives have been obtained using megazol as starting material and the heteroaryl hydrazine as key-intermediate. The hydrazone derivatives were obtained by acid catalyzed condensation of the hydrazine with the corresponding aromatic aldehydes in ethanol. The most active hydrazone compounds of this new series were the Brazilizone N (IC50=19.0uM) and 3-nitrophenyl derivatives (IC50=48.2uM), both being less potent than the Brazilizone A. Despite this fact, results obtained on some newly synthesized compounds, when used together with results from 1,3,4-thiadiazolylhydrazones previously reported, were essential in order to construct CoMFA model able to explain the stereoelectronic features required by the target bioreceptor in T. cruzi. From the 3D-QSAR studies, it is clear that the phenyl ring attached to the aryl-hydrazone moiety can bear bulky groups without jeopardizing biological activity, while ortho effects in general seem to have a deleterious effect to the biological activity. Additionally, 3D-QSAR CoMFA model was applied to understand the structural features involved in the trypanocidal activity of these analogues, giving insight for the design of new optimized anti-chagasic lead-candidates. CoMFA studies have shown that the steric and electrostatic requirements of their biorreceptor are clearly dependent on the phenyl ring orientation and on the steric and electronic characteristics of the substituents.

QT67 - Organostannic complexes with in vitro antimalarial activity against *Plasmodium falciparum* chloroquine-resistant parasites.

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Malaria treatment has been hindered by the limitations in the drug arsenal, so that the search of new antimalarials is needed to find alternative drugs. Our group has been involved in the search of new antimalarials based on ethnopharmacology for decades. We described the activity of several medicinal plants (reviewed by Krettli et al., M.I.O.C., 2001).

More recently, we described the activity of purified plant fractions, such as flavonoides from *Bidens pilosa* (P. R., 2004) and lignanes from *Holostilis reniformis* (A.A.C., 2007), from synthesized molecules following a rational approach, the case of phenazines from alfa and beta lapachol (B.M.C.L., 2004), and of chloroquine anologues (B.M.L., 2006). In the search of new compounds with antimalarial activivity we now tested organostannic complexes, which are compounds known for their byological interest as antifungal, antibacterial and biocide agents (E.J.M.C., 2005). A total of 11 organostannic compounds were evaluated for their antimalarial activity against the blood stages of *Plasmodium falciparum*, using a chloroquine-resistant (PfCR) strain W2. The methodology was based on the incorporation of hypoxanthine by the parasites after them being exposed to the test drugs and controls for 24 hours. The amount of hypoxantine incorporated by the live parasites enabled the determination of drug-response cycle inhibition. The inhibition of PfCR growth by the molecules was measured in relation to controls without drugs by levels of [3H]-hypoxanthine uptake. At 25μg/ml, 9 of the 11 complexes were active causing reduction of 84-64% parasitemia. The next step of the work is to establish the half-maximal inhibitory response (IC50) for these 9 complexes, to test their in vivo activity against *P. berghei* and determine toxicity in vitro to human hepatoma cells. Financial Support: CNPq and FIOCRUZ

QT68 - Antimicrobial activity of bornyl benzoates against *Trypanosoma cruzi* epimastigotes

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American trypanosomiasis or Chagas' disease is a chronic disease caused by the parasite *Trypanosoma cruzi*. Specific chemotherapy with benznidazole or nifurtimox has been recommended for treatment of acute and congenital infection, as shown by the clearance of parasitaemia and the disappearance of antibodies to *T. cruzi* (negative seroconversion). In this work, the *in vitro* effects of two synthetic 2-bornyl benzoates on growth and ultrastructure of epimastigote forms of *Trypanosoma cruzi*, were investigated. The discovery of new drugs for the treatment of Chagas' disease is a novel goal facing the researchers. We were reporting two compounds that exert an antiproliferative effect on the epimastigote forms of *Trypanosoma cruzi Dm*28c strain. The IC_{50}/72 h of 1,7,7-trimethyl-bicycle[2,2]heptan-2-yl 3',4',5'-trimethoxy-benzoate (**R7**) and 1,7,7-trimethyl-bicycle[2,2]heptan-2-yl benzoate (**R8**) were 10.1 μg/mL and 12.8 μg/mL, respectively. In addition, toxicity of the compounds was evaluated on human larynx carcinoma (HEp-2) cells, by using the dimethylthiazol diphenyl tetrazolium bromide (MTT) method. The cytotoxic concentrations of R7 and R8 on HEp-2 cells were 683.0 and 629.0 μg/mL, respectively. Observation by transmission electron microscopy of treated epimastigotes showed extensive intracellular damage including vacuolization, degradation of Golgi apparatus and the presence of myelin-like figures. Some of the vacuoles in the R7 treated cells appeared to contain cytoplasmic components. In addition, a cellular aggregation with possible parasites' membrane fusion was observed only after R7 treatment. Our studies demonstrate for the first time the *T. cruzi*-antiproliferative activities of 2-bornyl benzoate synthetic derivatives. Supported by CAPES, DECIT/SCTIE/MS - CNPq, MCT, PROPPG-UEL.

Vetores - Vectors

VE01 - Initial study of seasonality of tabanids (Diptera: Tabanidae) in the area of the Planato Catarinense, Santa Catarina, Brazil

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Introduction: The tabanids are considerate an important mechanical transmitters of several pathogenic agents to livestock's in South America, among them, the *Trypanosoma vivax* and the *Trypanosoma evansi*. In the South of Brazil were described and confirmed cases of tripanosomiasis by *T. evansi* in the state of Rio Grande do Sul, as well as suspicious cases of *T.vivax* or *T. evansi* in Santa Catarina. The objective of this study is to determine the composition and the population dynamics of the tabanids in the area of the Planalto Catarinense that leads the cattle breeding in the state of Santa Catarina, and can be an important place in an epidemic outbreak. The study is being driven monthly by collections of tabanids that land spontaneously in animals baits, at Haras Tessarolo, a property that has the creation of bovines, ovines and equines. Results: Until the moment were identified the genera *Catachlorops* sp., *Phaeotabanus* sp. and *Chrysops* sp., however parasites were not found inside. Other specimens are being taxonomic characterized and evaluate as for possible infection. This is the first work of tabanids description done in Santa Catarina, Brazil.

VE02 - SALIVARY GLAND MORPHOLOGY OF *Thyrsopelma guianense* (DIPTERA:SIMULIIDAE), MAIN VECTOR OF ONCHOCERCIASIS IN THE BRAZILIAN AMAZON.

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Blackflies are vectors of *Onchocerca*, agent of onchocerciasis. *Thyrsopelma guianense* is the main vector in a single Brazilian foci (Yanomami Indian area). The salivary gland is responsible for production and secretion of saliva which helps in blood feeding and transmission of *Onchocerca*. The aim of this study is to characterize the salivary gland of the *T. guianense* by laser confocal microscopy (LCM). Our results showed that the salivary gland is a single organ composed by two identical units, the tubular lobes,each one composed of three regions (proximal, medial and distal). Also, each lobe contains one glandular part with a connected structure to it that needs further investigataion (probably a saliva reservoir). The saliva production and secretion was observed only the in medial and distal regions of the two lobes. It appears that the saliva accumulation starts after 24 h after emergence with a peak at 48 h. In other physiological ages it was observed a decrease of saliva production and secretion. Our analysis by fluorescent lectin labelings (Con A, LPA, HPA, PNA and BS1) associated to FITC-Phalloidin (actin marker) and DAPI (nuclear marker) showed the salivary gland profiles at different physiological ages. The cytoskeleton defined the acinar aspects of the gland lobes with the presence of a simple secretory epithelium. The nuclei of the secretory cells were evidenced with DAPI staining. Finally, the lectin labelings showed that the salivary glands differ in intensity of each sugar production according to age. This work is the first detailed observation of the salivary gland of *T. guianense*. However, more studies are necessary to elucidate the sugar composition and their relation with the production and secretion of saliva of this important vector of the *Onchocerca*. Financial support: CNPq, FAPEAM/CAPES, FIOCRUZ and Mineração Taboca S/A.

VE03 - Study on Phlebotomine Sand Fly (Diptera: Psychodidae) Fauna in Contendas do Sincorá, State of Bahia, Brazil

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Phlebotomines are wild insects that are approaching the human environment, developing a close relationship with human beings and their domestic animals. The *Lutzomyia* genus has great importance on public health in the New World, with some species implicated in the transmission of causal agent of leishmaniasis, bartonellosis and arboviruses. The species of *Leishmania sp.* that parasite man present several different natural reservoirs and vectors, possibly due to the straight relation between sand flies species and their feed source. The aim of the study is to identify potential leishmaniasis vectors among the phlebotomine fauna. Systematic captures were performed twice every month in the municipality of Contendas do Sincorá, southwest area of Bahia State using HP light traps from June 2005 to June 2006. Sand flies captures were using for fauna study and too the females were used for natural infection examination, by polymerase chain reaction. Eleven different species of phlebotomine were found among a total of 1933 captured specimens. The highest percentage of individuals was collected in the peridomicile (54.6%) against in the intradomicile (41.3%). *Lutzomyia longipalpis* was the most common species, followed by *L. evandroi, L. intermedia, L. capixaba, L. lenti, L. termitophila, L. whitmani, L. migonei, L pessoai,*

L. sordelli and *L. oswaldoi.* No Leishmania DNA was present in any of the specimens tested. A better understanding of sand fly fauna in endemic area may contribute to the design of better control measures to decrease transmission.Support: FAPESB, CNPq/FIOCRUZ (PAPES).

VE04 - Study on Phlebotomine Sandfly (Diptera: Psychodidae) Fauna in area of transmission of American Cutaneous Leishmaniasis in Jequié, State of Bahia, Brazil.

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Leishmaniasis is a disease induced by a protozoan parasite and transmitted by sandfly. The *Lutzomyia* genus has great importance on public health in the New World, with some species implicated in the transmission of causal agent of leishmaniasis, bartonellosis and arboviruses. The aim of the study is to identify potential leishmaniasis vectors among the phlebotomine fauna. A study of phlebotomine sandfly fauna was carried out in an endemic area of American cutaneous leishmaniasis (ACL) in the municipality of Jequié, southwest area of Bahia State. Captures were performed with HP light traps in 5 differents captures sites, every month (August 2005 to November 2006). A total of 1143 sandflies were captured and identified. Four species were found, belonging to genus *Lutzomyia.* The highest percentage of individuals was collected in the peridomicile (98.2%) against in the intradomicile (1.5%). *L. intermedia* (49.2%) and *L. whitmani* (44.1%) was the most common species, both insects vectors of ACL, followed by *L. migonei* (4.3%) and *L fischeri* (2.4%). Understanding the population dynamics of sandflies species may contribute to the design of better control measures to decrease transmission of *L. braziliensis* and consequently the incidence of leishmaniasis. Support: FAPESB, CNPq/ FIOCRUZ(PAPES)

VE05 - Factors affecting the distribution of Lutzomyia longipalpis in an urban focus of visceral leishmaniasis

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Visceral leishmaniasis (VL), caused by Leishmania infantum and transmitted by Lutzomyia longipalpis is a growing problem in many Brazilian cities. Although little is known about the factors that permit the vector to become established in urban situations, these insects require terrestrial breeding sites (friable soil, humid and rich in organic matter) and diurnal resting sites (humid, protected from direct sunlight), as well as sources of sugar(plants) and bloodmeals (vertebrates, including reservoirs of Le. infantum). Information on these factors could be used in vector control through environmental modification to reduce Le. infantum transmission. In this study we are attempting to correlate presence of Lu. longipalpis with environmental factors related to the vital requirements mentioned. Eighty-six houses were selected in the Satelite and Vila Bandeirantes neighbourhoods of Teresina-PI. Sand fly sampling is carried out using sticky traps 10*house* hung for one week. Numbers of sand flies captured per house are noted and correlated with the following variables:(a) % vegetation cover/species of plant present (b) type of animal shelters (d) sanitation (presence of open drainsouthouses and piles of building materials in yards) (e) soil characteristics (pH, friability, organic content) and (f) numbers of each species of potential bloodmeal source. Numerical parameters will be regressed against numbers of sand flies ,R^2,while nominal variables will be analysed by X^2 test. Partial results obtained include the following: 5 Lutzomyia species have been collected to date (97.3% Lu. longipalpis) in 51 houses (59.3%). There are 12 species of potential bloodmeal sources, most abundant being chickens (n = 953), humans (445) and dogs (118).Vegetation coverage varies from 0.65 - 6.36%. Soil organic content ranges from 0.71 - 11.17% and pH from 5.5-9.8. Piles of building materials are present in 50% of yards and 67% have outhouses/open drains.

VE06 - Real-time PCR application for the diagnosis, quantification and discrimination of *Leishmania* spp in *Lutzomyia* vectors captured in different endemic regions of Brazil

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In the last years, cutaneous (CL) and visceral (VL) leishmaniasis have expanded for most Brazilian regions and are now spreading to the periphery of urban cities, constituting a serious health problem. *Leishmania (V) braziliensis* is responsible for more than 26,000 annual human CL notifications and *L (L) chagasi* enrolled with VL autochthonous cases. Despite the important role of *Leishmania* in the scenario of tropical diseases, current knowledge concerning the identification of natural infection in sandfly vectors from Brazilian endemic areas is poor for conventional parasitological diagnosis and virtually nothing has been performed using molecular tools. This study aims the development of a real-time PCR (SYBR-green) assay targeting *Leishmania* kDNA minicircles to simultaneously discriminate between species and quantify parasite load in *Lutzomyia* sandflies captured in Brazilian localities previously selected by their distinct endemic profiles

and presenting recent leishmaniasis notifications. The kDNA isolated from *L. braziliensis* and *L. chagasi* promastigotes were used in ten-fold dilutions as standard samples for absolute quantification. The test detected DNA concentration equivalent to 10^{-2} parasite/reaction in five independent runs for each kDNA source, demonstrating high reproducibility and sensitivity. Although both *Leishmania* kDNA amplicons present 120 bp, the dissociation curve analysis was able to distinguish the two parasite species based on their T_m differences. These data were confirmed by cloning and sequencing the amplified products, attesting that the minicircles conserved region from *L. chagasi* (higher T_m, 81.5^{o}C) has, in general, the addition of one cytosine compared to *L. braziliensis* (lower T_m, 79,3^{o}C). Sequence alignment revealed a higher G/C content (49,6%) in *L. chagasi* relative to *L. braziliensis* (48,3%). The results suggest the potential use of SYBR-green methodology to discriminate *Leishmania* species circulating in distinct endemic areas from Brazil, besides its capacity to correlate parasite load in sandfly vectors with the endemic levels of the studied areas. Support: CAPES/CNPq

VE07 - FREQUENCY OF SINANTROPIC TRIATOMINES IN BERILO MUNICIPALITY, JEQUITINHONHA VALLEY, MG, BRAZIL: ASPECTS OF THE EPIDEMIOLOGICAL SURVEILLANCE.

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The epidemiological surveillance is of great importance for the maintenance of the good results obtained until now for the Chagas disease Control Program (CDPC). In this context, the knowledge of the triatomine species, its dispersion and preference for intra or peridomiciliary environment is essential information to plan the control activities. Data of notifications received for the guardians of the local epidemiological surveillance, from October/2001 to January/2006, kept in the Regional Adminstration Health (RAH) from Diamantina, MG, were analyzed. In this period a total of 114 triatomines was sent to RAH/Diamantina and only 63 (55.3%) were examined. Of these, 2 (3.2%) were infected by *Trypanosoma cruzi*. The species of triatomine captured in the municipality were: *Pantrongylus megistus* (46.5%), *Triatoma pseudomaculata* (21%), *Panstrongylus geniculatus* (18.4%), *Panstrongylus diasi* (11.4%), *Triatoma vitticeps* (1.8%) and *Triatoma sordida* (0.9%). Seventy four percent of the captured triatomine was found in the intradomicile. In recent years, *T. pseudomaculata* specie is acquiring importance in the municipality, being in 2005, the most captured species in the intradomicile for the local inhabitants, although *P. megistus* remains being the most captured species in the global period (2001 - 2006). However *T. pseudomaculata* is progressively increasing its degree of antropofilia becoming close to the domiciliary units (DUs) and consequently a species of increasing epidemiological importance in the municipality. It was verified that one portion of the triatomines arrives in RAH/Diamantina without conditions of to be examined, probably due the delay of the guardian visits to the Triatomine Information Post (TIPs) or the bad way of capture carried out by the inhabitants. The observed data strengthen the importance of the epidemiological surveillance in Berilo since that native and ubiquitous species always will offer risk to the exposed population. **Financial support: FAPEMIG, CNPq, SESU/MEC, PROEX/UFOP.**

VE08 - Leeches: could they act as the biological vectors of *Trypanosoma evansi* ?

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Trypanosoma evansi is the aethiological agent of Surra; it is mechanically transmited by biting flies (Tabanidae and Stomoxidae) and has the widest geographycal range of all the pathogenic trypanosome species. *Trypanosoma evansi* can cause disease in domesticated livestock in many countries of South America, Africa and Asia, but it was also described as infecting sylvatic mammals, such as: deer, ocelot, coatis and capybaras. Several foci of the infection of *T. evansi* have been described in flooded areas and such habitats also offer good conditions for leeches´ reproduction. Previously we have demonstrated the experimental infection of *Rattus novergicus* by a strain of *T. evansi* through mechanical transmission by leeches (Glossiphonidae*Haementeria* sp.) born in a laboratory colony. In the present study we are discussing the behaviour of the *T. evansi* in this leech species. From ten minutes to twenty four hours, it was observed stumpy and slender trypomastigotes as well as round forms randomly dispersed in the gut, here the parasites formed clusters, apparently with subsequent fusion of the clustered cells. It was also observed the invasion of salivary glands and in the proboscis the parasites were observed as intracellular forms. The observation of such round multinucleated forms that resembles the early stage of amphibian trypanosomes in their leech vectors, besides the invasion of epithelial cells as well as the salivary glands could represent some degree of adaptation of *T. evansi* in the leech. This possibility is supported by the fact that the invasion of salivary glands is also observed among others Salivaria in their biological vectors. Those results suggest that the leeches could have some role as a vector in the sylvatic cycle of the *T. evansi* and they could probably also be responsible for the transmission of the disease during the periods of low population density of tabanids.

VE09 - THE GEOMETRIC MORPHOMETRICS AS A TOOL TO MONITOR INVASIVE POPULATIONS OF TRIATOMA VITTICEPS (STAL, 1859) (HEMIPTERA, REDUVIIDAE, TRIATOMINAE)

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The invasive behavior of Triatoma vitticeps is of increasing concern to health authorities (National Health Foundation in Brazil- Funasa) throughout its geographic distribution in three States: Minas Gerais (MG), Rio de Janeiro (RJ) and Espírito Santo (ES). In ES the numbers of domiciliary invasions are significantly higher, increasingly reported in the last years, especially in the south of this State where several new cases of Chagas disease have been registered. Our objective is to understand whether the higher domiciliary invasion of T. vitticeps in the south of ES could be related to different genetic attributes and/or to some environmental pressures. As a first approach to explore the invasive behavior of T. vitticeps in south ES, we used geometric morphometry (GM) comparing specimens from there with specimens from other geographic areas. A total of 139 digital pictures of mounted (Permount®) wings were characterized at 13 landmarks (software TPS dig, Rohlf), and the resulting coordinates were submitted to Procrustean superimposition and thin plate spline analyses (software MOG, Dujardin) and discriminant analyses (software PAD, Dujardin). Size (.'centroid"size) and shape variables (both uniform"and .'non-uniform"components) were able to differentiate three populations. Size variation was in accordance with altitudinal changes. Shape was completely described by two discriminant factors: DF1 (77%) was in agreement with both geographic distances and altitude, and DF2 (23%) distinguished the southern ES population. A possible genetic source for this latter component of variation will be examined by mitochondrial DNA sequencing analyses, comparing the same specimens. Financial Support: CNPq

VE10 - Fitness studies of transgenic malaria-resistant mosquitoes

The introduction of genes that impair Plasmodium development into mosquito populations is a strategy being considered for malaria control. The effect of the transgene on mosquito fitness is a crucial parameter influencing the success of this approach. We have previously shown that anopheline mosquitoes expressing the SM1 peptide in the midgut lumen are impaired for transmission of Plasmodium berghei. Moreover, the transgenic mosquitoes had no noticeable fitness load compared with nontransgenic mosquitoes when fed on noninfected mice. We have shown that when fed on mice infected with P. berghei, these transgenic mosquitoes are more fit (higher fecundity and lower mortality) than sibling nontransgenic mosquitoes. In cage experiments, transgenic mosquitoes gradually replaced nontransgenics when mosquitoes were maintained on mice infected with gametocyte-producing parasites (strain ANKA 2.34) but not when maintained on mice infected with gametocyte-deficient parasites (strain ANKA 2.33). These findings suggest that when feeding on Plasmodium-infected blood, transgenic malariaresistant mosquitoes have a selective advantage over nontransgenic mosquitoes. This fitness advantage has important implications for devising malaria control strategies by means of genetic modification of mosquitoes

VE11 - FEEDING INFLUENCE ON THE DEVELOPMENT OF *TRYPANOSOMA CRUZI* IN THE DIGESTIVE TRACT OF *TRIATOMA BRASILIENSIS* (HEMIPTERA: REDUVIIDAE)

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Chagas disease is an important parasitic infection in Latin America, affecting 16-18 million people. *Triatoma brasiliensis* is one of the main vectors in the semiarid areas of Northeastern Brazil, successfully colonizing natural and artificial ecotopes, mainly human dwellings. The development of *T. cruzi* in the digestive tract of the triatomines is influenced by feeding and starvation periods of the insect. During the blood feeding on infected vertebrate hosts, the ingested blood trypomastigotes develop to epimastigotes, which later, intensively multiply in the posterior midgut. The metacyclogenesis occurs in the rectum of the insect. During the feeding, the flagellates are released into the urine and feces of the infected bugs. In the present work, we investigated the development of the *T. cruzi* isolate (MDID/BR/1994/C48), which was obtained from *Philander frenata* and already classified as TcI genotype. The triatomines were fed and dissected at different days after feeding (3, 5 and 10 daf). According to the previous results, *T. cruzi* (C48 isolate) colonized mainly the rectum, preferentially the lumen, with a high population density of parasites at 5 daf. A high number of parasites was also observed at 5 daf, in the rectal wall. The number of flagellates was reduced at 5 and 10 daf in the small intestine region. Therefore, further investigations will be done to evaluate the stages and metacyclogenesis of the life cycle of *T. cruzi* (C48) strain in the digestive tract of *T. brasiliensis*. *Key words*: Digestive tract, *Triatoma brasiliensis*, *Trypanosoma cruzi*, starvation Financial Support: FAPERJ (E-26/170.122/2004), CNPq (472276/2006-9) and FIOCRUZ.

VE12 - Phosphate content could be important for development of *Trypanosoma rangeli* in the invertebrate host *Rhodnius prolixus*

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The intestinal tract of many insects is colonized by trypanosomatids. Especially in heteroxenous trypanosomatids, the causative agents of many diseases in vertebrates and plants, different developmental stages occur in different regions of the intestine. *Trypanosoma rangeli* is a South American trypanosoma, considered harmless to humans and animals although can infect triatomine insects. After the ingestion of trypomastigote forms during the blood meal, *T. rangeli* differentiate into short epimastigotes, proliferate in digestive tract, across the intestinal barrier and achieve haemolymph, where long forms are founded. Parasites complete your development in the lumen of salivary gland of insect, where metacyclogenesis takes place. Inorganic phosphate is an important nutrient to all cellular functions. The phosphate is an essential compound to the cell growth and production of many cellular components as nucleic acids, lipids, sugars and others. Ecto-phosphatases are enzymes that present your catalytic site faced to external medium and have been detected in several microorganisms, including *T. rangeli*. In order to evaluate the importance of inorganic phosphate and phosphatase activity to the development of *T. rangeli* in its invertebrate host *Rhodnius prolixus*, such as cell proliferation, differentiation and parasite-host cell adhesion, we measured the inorganic phosphate content and the phosphatase activity in *R. prolixus* hemolymph, crop, midgut and rectum. The data showed that phosphate content was higher in the crop with values of approximately 2,33, 1,13, 0,21, 0,096mmols Pi x mg^{-1}ptn for crop, midgut, rectum and hemolymph, respectively. The phosphatase activity had values of approximately 46,81, 32,44, 3,51, 1,91nmols Pi x h^{-1} x mg^{-1} ptn for crop, midgut, rectum and hemolymph, respectively. We also evaluated the effects of the homogenated tissues of *Rhodnius prolixus* in the ecto-phosphatase activity of *T. rangeli*. The results show that rectum stimulated significantly *T. rangeli* ecto-phophatase activity. Supported by CNPq and CAPES

VE13 - Phylogenetics relationships within the *Triatoma sherlocki* Papa, Jurberg, Carcavallo, Cerqueira & Barata, 2002 and *T. brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae) using on sequencing of the nuclear and mitochondrial genes.

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Mitochondrial (cytocrome b and 16S) and nuclear (ITS-2 and 28S) gene sequencing of *T. sherlocki* and *T. brasiliensis* was carried out with the aim of learning more about the species and its phylogenetic relationship to the species of the Triatominae subfamily. The phylogeny based on the two mitochondrial genes disclosed that *T. sherlocki* and *T. melanica* were closely related and that *T. brasiliensis* was a sister species of the two, with the exception that in for the Parsimony analysis of the 16S gene, *T. melanica* appeared as a sister species of the clade formed by *T. sherlocki* and *T. brasiliensis*. The species *Panstrongylus megistus* and *T. infestans* are related to North American species in phylogenetic analyses of the 16S mitochondrial gene and the amino acid sequences of cytocrome b, respectively. The phylogeny based on the 28S nuclear gene revealed to a polytomy involving *T. sherlocki*, *T. brasiliensis* and *T. melanica*. This phylogeny was underestimated due to the small number of species present in the tree. This same relationship occurred in the phylogeny of ITS-2 nuclear gene, in analysis based on Maximum Parsimony. The analysis of distance in this gene revealed proximity between *T. sherlocki* and *T. brasiliensis*, with *T. melanica* as sister species. This phylogeny showed *T. maculata*, *T. brasiliensis* and *T. infestans* to be closely related to North American triatomines. Certain species, such as *T. infestans* and *P. megistus*, exhibited more than one topology with different genes, and further study is needed to define the phylogenetic positions of these species. The proximity of *T. sherlocki* to the *T. brasiliensis* haplotypes, especially with the species *T. melanica*, may reveal a possible phylogeography originaling from *T. brasiliensis* ancestors, thus implying derived species. Support: FAPESP (05/52608-6 and 2006/02778-5) and FUNDUNESP (066/06).

VE14 - Molecular Phylogeography and applications in the biogeography of Chagas disease vectors *Rhodnius pictipes* (Hemiptera: Reduviidae) in the Amazonian basin

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For many years, the epidemiological relevance of silvatic Chagas disease vectors has been underestimated. This is particularly important in the Amazon region, where there are no domestic vectors (with the exception of *Triatoma maculata* in Roraima), and which has seen a recent increase in disease prevalence. *Rhodnius pictipes* is one of the most widespread triatomine species in that region. Although silvatic and unable to colonize homes, at night this species (often infected with *Trypanosoma cruzi*) might invade human habitations attracted by artificial light, in forest fringe areas (especially in Pará and Amazonas), leading to disease transmission.

There is no doubt that deforestation plays an important role, in terms of favoring this synanthropic behavior, by disrupting the natural zoonotic cycle. Given the emerging status of Chagas disease in the Amazon, it becomes relevant to understand the population structure of *R. pictipes* in that region. In order to determine the phylogeographic pattern and current distribution of *R. pictipes*, a total of 120 samples from 10 populations will be sequenced for two gene fragments (682bp of the mitochondrial cytochrome *b* gene, and 700bp of the nuclear ribosomal internal transcribed spacer, ITS-2). To date, 47 samples from six locations have been sequenced for the cyt *b* gene fragment. Sequence alignment revealed 12 polymorphic sites that comprise six haplotypes. Phylogenetic analysis of such sequences (Neighbor Joining tree with Kimura 2parameter distances) indicates the existence of three genetic groups occurring in the states of Amazonas and Roraima, in Brazil, and in French Guyana. Since the distribution of *R. pictipes* overlaps that of the *R. robustus* complex, a biogeographic study in the Amazon will be performed to investigate whether the former species reveals the same signature (i.e. deep subdivisions indicative of incipient speciation) detected in *R. robustus*, as expected if both experienced the same past vicariant events.

VE15 - INNATE CELLULAR DEFENSE OF HEMOCYTES FROM *Aedes aegypti* AND *Anopheles aquasalis* : PRELIMINARY RESULTS

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Innate immunity in insects is composed of both cell-mediated and humoral immune responses. In insect cellular immune responses, hemocytes participate in phagocytosis, nodule formation and encapsulation, whereas synthesis of antimicrobial peptides and activation of prophenoloxidase are important humoral immune responses. Hemocytes from adult *Ae. aegypti* were recently classified into prohemocytes, adipohemocytes, granulocytes, plasmatocytes, oenocytoids and thrombocytoids. Far less is known about the hemocytes produced by anopheline mosquitoes including *An. aquasalis* that is vector of human malaria in South America. Our knowledge about the immune responses of insect disease vectors is still limited. Several reports exist regarding the participation of mosquito hemocytes in defenses responses to foreign organisms. The types of hemocytes involved and their function in mediating these responses, however, are unclear. The aim of the current study was identify which hemocytes are involved in phagocytosis of foreign particles using laser scanning confocal microscopy. Phagocytosis assays were conducted by injecting fluorescent conjugates like ovalbumin (FITC-OVA), FITC-latex beads and FITC-lectins into the thorax of cold anesthetized mosquitoes. After 30 minutes, the hemolymph from 50 insects were collected directly in a 4% paraformaldehyde fixative solution. All the samples were also stained with DAPI for nucleus visualization. In *Ae. aegypti* we found that granulocyte are involved in phagocytosis of latex beads and HPA lectin. It was clearly visualized the position of the FITC labeled latex beads next to the nuclei of cell. However, in *An. aquasalis* the granulocyte and plasmatocyte are involved in responses against FITC-OVA. Additional experiments are being developed to better identify aspects of the mosquito hemocyte phagocytosis. Such knowledge will contribute to understand the cellular immune response of mosquito vectors against pathogens. Acknowledgements: The financial support of CNPq, PRONEX, FIOCRUZ and FAPEMIG.

VE16 - *AEDES* INNATE IMMUNE RESPONSES TO VIRUS INFECTION

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Diseases caused by arthropod-borne virus are significant public health problems, and novel control methods are needed to block pathogen transmission. Although *Aedes aegypti* is the main vector of both yellow fever and dengue virus, little is known about mosquito responses to virus infection. Innate immune responses are mediated by activation of signaling pathways, in response to specific cell-pathogen interactions. In *Aedes aegypti*, it was shown that infections by fungi and gram-positive bacteria activate mostly toll pathway, through NF-κb related transcription factor Rel 1, while gram-negative bacteria activates IMD pathway through another NF-κb related transcription factor, Rel 2. In this work we analyzed the involvement of the three major mosquito immune pathways in response to sindbis and dengue virus infection. Both viral infections triggered an increase of Rel 1, Rel 2 and STAT relative expression in *Aedes aegypti* mosquitoes and *Aedes albopictus* C6/36 cell line. In addition, blood fed mosquitoes presented higher levels of these transcription factors when compared with sugar, BSA or latex-fed mosquitoes, indicating that blood-induced activation of innate immune pathways can be an important factor in pathogen invasion and vector competence. Supported by CNPq, HHMI, Pronex and FAPERJ.

VE17 - LIPID METABOLISM OF *Herpetomonas megaseliae*

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Herpetomonas megaseliae is a monoxenic flagellated parasite, a member of Trypanosomatidae family, which infects

the dipteran *Megaseliae scalaris*. During its cycle of life, the parasite keeps in the lumen of the insect intestine. *H. megaseliae*, although nonpathogenic to humans, is a safe experimental model for biochemical studies of lipid metabolism of trypanosomatides. Lipids are hydrophobic molecules that play a large variety of cellular functions of great importance for all organisms. In this work, we have analyzed the capacity of *H. megaseliae* to incorporate and metabolize lipids. The parasites were incubated with ^{3}H-fatty acid for 5 and 240 minutes for 28°C. After the incubation times, the cells were washed with PBS 1% sucrose and subjected to lipid extraction. Labeled lipids were separated by thin-layer chromatography (TLC) for neutral lipids. The spot of each lipid was scraped from the TLC plate and the radioactivity associated was estimated by scintillation counting. The results showed that, after only 5 minutes of incubation, the radioactivity was mostly associated with phospholipids and diacylglycerol. After that, the cells were also able to produce triacylglycerol from ^{3}H-fatty acids. In the future, experiments using ^{14}C-acetate and ^{32}Pi as precursors will be performed to gain more insight about *H. megaseliae* lipid metabolism. This work was supported by CNPq, FAPERJ and IFS.

VE18 - *Trypanosoma rangeli*-derived GIPLs down-regulate *Rhodius prolixus* salivary gland Nitric Oxide Synthase expression

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Rhodnius prolixus is a blood-sucking bug whose saliva contains several antihemostatic molecules. One of such compounds is a vasodilatatory gas named Nitric Oxide (NO). NO production is catalized by a Nitric Oxide Synthase (NOS) present in this insect's salivary glands. It has been demonstrated that *Trypanosoma rangeli* infection impairs *R. prolixus* salivary gland NO generation (Garcia et al, 1994). The mechanisms involved in the regulation of NO production during both salivary gland development and infection have not been characterized, so far. Using western blotting techniques, immunohistochemical analysis and a NO fluorescent probe (DAF-2A diacetate, Molecular Probes), we have observed that *T.rangeli* reduces *R. prolixus* NOS levels and NO generation. Parasite surface glycoinositolphospholipids (GIPLs) were administrated to uninfected bugs in order to determine its possible role in the manipulation of host NOS activity. Insects were injected with *T.rangeli*, or *Phytomonas serpens*-derived GIPLs or with *Trypanosoma cruzi* mucins . *T.rangeli* -derived GIPLS decreased NOS-associated NADPH-diaphorase activity and NO production, therefore partially mimicking infection. However, no effects were observed when *P. serpens*-derived GIPLs or *T. cruzi* mucins were administrated to the insects. Preliminary results indicate that *T.rangeli* GIPLs also regulates salivary gland protein phosphorylation profile in the same pattern as we have observed during *T.rangeli* infection. In further experiments we have administered PMA to uninfected animals and observed a 50% increase in NOS-associated NADPH activity. In conclusion these results suggest that parasite invasion in bug salivary glands induces a downregulation of NO production through a decrease on NOS synthesis which is initially triggered by its surface molecules and involves the intracellular handling of protein kinase C activity. Supported by CNPq, FAPERJ, IFS.

VE19 - Complement inhibition in the anterior midgut (crop) of the Triatominae *Triatoma brasiliensis*.

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Various biological activities have been described in the saliva of haematophagous insects, one of these is the inhibitory activity upon the complement system. This activity was already described by our research group in phlebotomine sandflies (*Lutzomyia longipalpis* and *L. migonei*) as well as in some triatomines (*Triatoma brasiliensis*, *T. infestans*, *Rhodnius prolixus* and *Panstrongylus megistus*). It is probably that, in haematophagous insects this activity is involved with protection of the midgut epithelia against the complement system. The objective of this work was to investigate if the anterior midgut (crop) of *T. brasiliensis* also possess an inhibitory activity against the complement system as well as determine the point in which the classical and alternative pathways are interrupted. The employed methodology is based on the fact that the human complement is triggered by contact with antibodies (classical pathway) or agarose adhered to a microplate. The components of the system which are activated in cascade and become adhered to the activator surface (for example the components C3b and C4b) can be identified by mean of specific monoclonal antibodies. An inhibitory event reduces the number of adhered components downstream the point of the cascade affected. The results showed that the intestinal contend of *T. brasiliensis* was capable to reduce the deposition of C3b as well as C4b in the alternative and classical pathways respectively. The interruption of the complement cascade in its initial events as observed here can be very important to improve the efficiency of the intestinal inhibitory molecules. At the moment, we are working in the identification and characterisation of the salivary as well as intestinal molecules responsible for the complement inhibitory activity. Supported by CNPq and FAPEMIG.

VE20 - Lipase activity in *Aedes aegypti* midgut: Characterization and *in silico* search

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The great amount of lipids present in blood meal is a relevant source of substrates for oogenesis in female mosquitos. However, information about lipid digestion is scarce despite the importance of this process in lipid metabolism. Enzymes with triacylglycerol-lipase activity probably have an essential role, digesting triacylglycerols and generating free fatty acids that will be incorporated by the midgut cells. The study of digestion mechanism of triacylglycerol is important for the understanding of the regulation of lipid absorption in midgut of *Aedes aegypti*. The lipase activity present in the mosquito midgut was characterized with the use of homogenized organs. Enzyme activity was linear until 80 min of incubation. Lipase activity was affected by pH variation and showed optimum activity values at pH 7.5. Ionic strength and calcium concentration also affected lipase activity, which was maximal in the presence of 0.5 M NaCl and 1 mM Ca^{2+}. It was inhibited by PMSF, and it was 10% of control at 10 mM PMSF. *Aedes aegypti* genome has 41 genes annotated as codifying lipases. Analysis of these sequences in Prosite software showed that only 16 of the 41 putative proteins have the lipase conserved motif of active site. SignalP software analysis showed that 9 of the remaining 16 proteins have a signal peptide to be sent to secretory pathway, and TMHMM software analysis detected a putative transmembrane domain in one of these proteins. In that way, there are 8 candidate proteins to be digestive lipases in *Aedes aegypti* midgut. Expression analysis will be necessary to identify which of these eight genes are indeed expressed in the midgut. Supported by CAPES, CNPq and FAPERJ.

VE21 - Interaction of *Blastocrithidia culicis* with *Aedes aegypti* midgut proteins

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The aim of this work was to study the life cycle of monoxenic trypanosomatids in insect hosts, and we used *Blastocrithidia culicis-Aedes aegypti* as a model. Our group demonstrated that *B. culicis* is able to survive for a long time in the midgut of *A. aegypti*, an important vector of human diseases such as dengue and yellow fever. The process of protozoa-midgut interaction occurs first with the flagella binding to the microvilli of the insect midgut cells followed by insertion of the flagella between the tight junctions of these cells. Here, we have identified the midgut proteins responsible for the *B. culicis* binding. Mosquito midgut proteins were analyzed by blotting assays using *B. culicis*-biotinylated-epimastigotes as a ligand. The development of this system with ECL showed that *B. culicis* bound to seven midgut proteins with molecular mass varying from 10 to 50 kDa. All these mosquito midgut proteins are glycoproteins, as they were recognized by lectins specific for N-acetyl-galactosamine, N-acetyl-glucosamine, L-fucose and galactose. In order to analyze biotin-labeled *B. culicis* proteins, total epimastigote extract was resolved by SDS-PAGE, blotted to nitrocellulose, incubated with avidin and then developed by the ECL system. Some *B. culicis* proteins were labeled with biotin but the protozoan parasites also presented naturally biotinylated proteins. To further investigate the expression of the biotinylated proteins, epimastigotes were treated with avidin-FITC and analyzed by flow cytometry. Our results show that midgut proteins that recognize *B. culicis* are glycoproteins. We also suggest that naturally biotinylated proteins were not expressed on the protozoan cell surface. Supported by: CNPq, FAPERJ, CAPES, #F.A. Dias is a recipient of "FAPERJ Nota 10" fellowship.

VE22 - Anticoagulant activity of *Lutzomia longipalpis* saliva

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Blood coagulation results from a complex series of zymogen activation reactions that form part of the mechanisms of host defense to injury. Haematophagous arthropods have evolved potent pharmacological substances that are secreted from the saliva into the feeding site. Therefore, salivary glands from blood-feeding insects are rich sources of antihaemostatic molecules. In a previous study, six members of a family of putative anticoagulants have been identified through massive DNA sequencing and proteomic analysis of the salivary glands of the sandfly *Lutzomyia longipalpis*, the vector of *Leishmania chagasi*. In this work we describe the anticoagulant activity of *Lu. Longipaplis*. Assays using extracts of salivary glands showed a delay in the coagulation time of human platelet-poor plasma, indicating and confirming anticoagulant activity. Clotting assays specific for of inhibitory effect by salivary gland was detected in assays that include factors of the intrinsic (Activated Partial Thromboplastin Time) or extrinsic pathways (Prothombin time), demonstrated that only the former was inhibited. Therefore, assays using purified proteins demonstrated potent inhibition of FX activation by the tenase complex (factor IXa/factor VIIIa/phospholipids) as well as prothrombin activation by the prothrombinase complex (factor Xa/factor Va/phospholipids). A better understand of the inhibitory factors that affect blood coagulation cascade by sandfly saliva may provide novel antihemostatic molecules. Supported by the CNPq, FAPERJ and FUJB

VE23 - INHIBITORY EFFECTS OF D-GALACTOSE ON ECTO-PHOSPHATASE ACTIVITY OF SALIVARY GLANDS OF Rhodnius prolixus.

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Salivary glands of Rhodnius prolixus represent a target organ of Trypanosoma rangeli metacyclogenesis. Some authors have observed a high number of parasites, mostly long epimastigotes, adhered to their outer surface. The invasion seems to be mediated by specific receptor-ligand interactions. In the present study, we investigated ecto-phosphatase activities on the surface of R. prolixus salivary glands. This enzyme is able to hydrolyze phosphorilated substrates in the extracellular medium. We have characterized phosphatase activities on salivary glands surface of R. prolixus and demonstrated its modulation by carbohydrates as D-galactose. Salivary glands present a lower level of hydrolytic activities, 4.3±b0.35 nmol p-NP x h-1 x gland pair-1. However, salivary glands incubated with 250mM of D-galactose showed a significant inhibition of ecto-phosphatase activity, 1.4±b0.78 nmol p-NP x h-1x gland pair-1. This effect was also observed on the ecto-phosphatase activity of short and long epimastigotes of T. rangeli, 0.7±b 0.08 and 0.7±b0.05 nmol p-NP x h-1 x 10^7 respectively. Regarding ecto-phosphatase activity of salivary glands incubated with long epimastigotes in the presence of D-galactose, the in vitro interaction assays demonstrated a reduction from 5.1±b0.9 to 2.9±b0.4 nmol p-NP x h-1 x gland pair-1x 10^7 compared to control. These data can be useful to understand the possible involvement of ecto-phosphatase in host-parasite interactions and/or reception and transduction of external stimuli.
Supported by FAPERJ-FIOCRUZ and CNPq

VE24 - Saliva of *Rhodnius* spp reveals apyrase activity

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Blood-feeding arthropods are able to constraint barriers imposed by host defenses due to the presence of a wide range of antihemostatic factors in their saliva, including vasodilators, antiplatelet factors and anticoagulants. We report here apyrase activities in the saliva of *Rhodnius brethesi*, *Rhodnius milesi*, *Rhodnius pictipes* and *Rhodnius robustus*. These apyrases are Ca^2+ dependent only and their optimal activities occur at 37^{o}C and pH 8.3. To identify apyrase activities, salivary gland contents were submitted to SDS-PAGE enzimography without previous boiling or reduction of the samples. This experiment allowed the identification of about 44 kDa bands displaying both ATPase and ADPase activities. Moreover, we performed apyrase activity from *R. brethesi* salivary gland content in two-dimensional gel electrophoresis. The protein mediating apyrase activity was identified by mass spectrometry. In vitro platelet aggregation assays showed that the content of 0.5 salivary gland pair of *R. brethesi*, *R. milesi*, *R. pictipes* and *R. robustus* completely abolished platelet aggregation induced by ADP. The wide distribution of apyrases in the saliva of Chagas disease vectors indicate that these enzymes play important roles during blood feeding. Supported by CAPES and CNPq.

VE25 - Production and in vitro functional characterization of a recombinant kazal type peptide expressed in the salivary gland of the *Triatoma brasiliensis*.

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On introducing their mouthparts into the host skin looking for blood, the triatomines trigger several repairing physiological responses. To counteract these responses and facilitate the blood ingestion, the triatomines release saliva, which possess a great variety of antihaemostatic components. In order to better explore the salivary molecules of the triatomines, our group sequenced a salivary gland cDNA library of the *Triatoma brasiliensis*. Among the molecules identified, we found a sequence with homology to the vasotab, a kazal type peptide with vasodilatory function from the tabanidae *Hybomitra bimaculata*, suggesting that the sequence could be responsible for the vasodilatory activity from the saliva of *T. brasiliensis*. The aim of this work is to characterize the biological activity of the protein expressed in the salivary gland of the *T. brasiliensis* with homology to the vasotab. The sequence of the gene was cloned into the pET SUMO vector, which possess the LacZ operon and an histidine tag (6xHis) that was used to purify the recombinant protein using the Probond Purification System (Invitrogen). At the same time, the expression of the gene in the salivary glands was knocked down by RNAi using one injection of 10 μg of a dsRNA homologous to the sequence of the gene. The expression of the recombinant was confirmed by SDS-PAGE. Western blot with antibodies against *T. brasiliensis* crude saliva recognized the purified recombinant. Preliminary assays for biological activities in vitro indicated that the recombinant has no anticoagulant action. Assays using rat aorta pre-contracted with phenylephrine promoted an additional vasoconstriction. The injections of dsRNA promoted about

60% reduction in gene expression in the knockdown group when compared to the control. New tests are been carried out using the recombinant and the saliva of knockdown and control insects to confirm the activity of the peptide upon vessel contraction. CNPq, CAPES, FAPEMIG.

VE26 - Adipokinetic hormone-induced lipid Mobilization in the fat body of *Rhodnius prolixus*

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The fat body is the main site for storage of both glycogen and lipids in insects, as *Rhodnius prolixus*. Triglyceride (TG) is stored in fat body adipocytes as cytosolic lipid droplets. TG hydrolysis (lipolysis) is mediated by a TG-lipase that has been purified from the cytosol. The end product of insect lipolysis is *sn*-1,2-diacylglycerol (DG) that is released in the hemolymph and loaded into the hemolymph lipoprotein, lipophorin (Lp). In insects, Lps are the major hemolymphatic lipoproteins that carry and distribute lipids of many classes between the tissues involved in lipid absorption, storage and utilization. In the fat body, the lipolytic process is under hormonal regulation by the neuropeptide adipokinetic hormone (AKH) that is produced by corpora cardiaca. AKH action is comparable to that of glucagon in mammals. It contributes to hemolymph sugar homeostasis and is also involved in the mobilization of sugar and lipids from the fat body during energy-requiring activities. In order to investigate the presence of AKH in *R. prolixus* and its effect on the lipid metabolism, the insects were injected with 3 pmol of synthetic AKH (Leucin-AKH). After 30 minutes the hemolymph was collected and the fat body was dissected. These samples were subjected to lipid extraction and high performance thin-layer chromatography (HPTLC). The plates were analyzed by densitometry. We observed that the amount of triacylglycerol at the fat body decreases dramatically (30 fold) and, consequently, the amount of diacylglycerol in the hemolymph increases (2 fold). We demonstrated, for the first time, that the fat body of a haematophagous insect is able to respond to AKH indicating the possible presence of a similar hormone in *R prolixus* hemolymph. Supported by CNPq, Faperj, IFS

VE27 - Juvenil Hormone Modulates *Aedes aegypti* Midgut Protein Tyrosine Phosphatase.

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Juvenile hormone (JH) regulates several aspects of reproductive maturation in the adult female mosquito, including previtellogenic oocyte growth, proliferation of ribossomes in the fat body and development of follicle cells. Corpora allata, a pair of endocrine glands with nervous connections to the brain, synthesizes and releases JH. In *Aedes aegypti* mosquitoes, JH levels are low at adult eclosion, elevated in sugar-fed females and low again after a blood meal. High JH levels are required in recently ecloded mosquitoes to prepare the female for the first blood meal including its digestion and egg formation. Intracellular signaling events occur in mosquito tissues upon these changes but they remain mostly unknown. The objective of this study was to determine the effect of JH in a mosquito midgut tyrosine phosphatase (PTP). Females were dissected in different days after emergence and total midgut PTP activity was measured. A significant increase on the activity was observed two days after emergence and then it slowly decreases. In a following experiment, two day-old mosquitoes were treated with 500 nM JH or acetone, applied topically in abdomen. Twenty four hours latter, an increase on PTP activity was observed in mosquitoes treated with JH. Experiments to observe the phosphotyrosine phosphorylation profile during emergence and in JH-treated insects are being conducted and may identify relevant phosphoproteins committed with midgut remodeling to allow mosquito blood feeding. In conclusion, altogether this data suggests for the first time that JH may act on midgut remodeling through the manipulation of intracellular phosphorylation events catalyzed by PTP. Sponsured by OMS, CNPq and FAPERJ.

VE28 - *Aedes aegypti* Salivary Gland interaction with *Blastocrithidia culicis*

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The knowledge about trypanosomatid biological cycle in their insect vectors is mostly restricted to pathogenic parasites. Very little is known about the monoxenous trypanosomatid development in insects. Studying the development of *Blastocrithidia culicis* in *Aedes aegypti* we started to fill some of these gaps. Recently, we demonstrated that *B. culicis* was able to colonize the digestive tract and reach the hemocoel of *A. aegypti*. We then ask if this protozoan could interact with the salivary gland of the mosquito. To answer this question we studied the interaction of *B. culicis* with explanted mosquito salivary glands by scanning and transmission electron microscopy. Our results showed that *B. culicis* not only bind, by the flagella to salivary gland but also invade this organ. Next, using a ligand blot assay and biotinylated live protozoan we demonstrate that *B. culicis* bound to five glycoproteins presents in the salivary glands extracts. Following, we investigate the carbohydrate involvement in the *in vitro* binding process. Addition of L-Fucose, α-methyl-D-mannoside, N-acetyl-D-glucosamine, β

-galactose, lactose and fetuin to interaction *in vitro* was able to inhibit the protozoan bind to salivary gland. *B. culicis* carbohydrates surface expression was assayed by cytometry using fluorescent lectins and Concanavalina A was the lectin that stronger labeled the protozoa. Fluorescent lectins were also used to analyze the carbohydrate expression on the salivary gland basal lamina. Our results showed labeling of the basal lamina by the following lectins: Concanavalina A, specific for mannose; *Triticum vulgaris* (WGA), that bind to N-acetyl-D-glucosamine; *Glycine max* (SBA), that bind to N-acetyl-D-galactosamine and *Arachis hypogaea* (PNA) that recognize β-galactose. All together, these results suggest that a carbohydrate mediated *B. culicis* - salivary gland interaction. Supported by CAPES, CNPq, FAPERJ, #F. A. Dias is recipient of "FAPERJ Nota 10"fellowship

VE29 - Expression, proteolytic activity and immunolocalization of trypsin in *Lutzomyia longipalpis*

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We are studying *Lutzomyia longipalpis*, the main vector of visceral leishmaniasis in Brazil. In sandflies of the Old World midgut enzymatic activity during blood digestion is one of the obstacles which *Leishmania* must surpass to succeed in establishing infection. In *L. longipalpis* little is known about the effect of the digestion process over parasites. We have previously described two trypsin cDNAs of *L. longipalpis*. One (*Lltryp1*) has a constitutive and the other (*Lltryp2*) has a bloodmeal induced transcription pattern in bloodfed females. We have cloned and expressed a fragment of *Lltryp2* cDNA, and the recombinant protein was used to produce a polyclonal anti-trypsin antibody. We have used this antibody in immuno-localization studies, and our preliminary results indicate that the enzyme distributes through the cytoplasm of non-fed and blood-fed insect midgut cells. We have also used this antibody in Western blot experiments using whole insects. A band of approximately 30 kDa, as expected, is detected between 6 and 48 hours after blood ingestion. Enzyme activity assays using dissected guts suggest that *L. longipalpis* females at 48 hours after infection with *Leishmania chagasi* have lower trypsin activity than non-infected insects. We are presently studying in-gel protease activity of whole insect preparations at different times after feeding. Gel incubation in buffer solutions with different pH values may reveal the optimal range for proteolysis. Gel incubation with specific inhibitors will reveal the nature of the gut enzymatic activity. Financial support: CNPq and PAPES-IV - Fiocruz. Corresponding address: erichltioc.fiocruz.br.

VE30 - CHAETIC SENSILA OF THE ANTENNA OF TWO SANDFLY VECTORS OF CUTANEOUS LEISHMANIASIS: A LASER CONFOCAL MICROSCOPIC STUDY.

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Lutzomyia migonei and Lutzomyia ovallesi are sympatric species found in South America including in Venezuela where they are vectors of Leishmania braziliensis and Leishmania mexicana respectively. The sensilla are sensorial organs with diferent shapes and functions distributed across the insect body. In the antennae, they are responsible for recognizing stimuli involved with feeding, aggregation, mating and are also capable of noticing odors, humidity and temperature. The chaetic sensilae is a long structure with spine shape inserted in the insect antennae. In the apical region, this sensilae has several porous revealing its sensorial-mechanical functions important for host searching. The present study developed a methodology to observe morphological details of the chaetic sensilae of the two insect vectors under the confocal laser microscopy. We used fluorescent lectins (WGA, ConA, BS1, PNA, HPA, RCA) to label sugar residues, phaloidin to mark the actin cytoskeleton, vital stain fluorescein and PKH-26 fluorescent cell marker to recognize the cell populations and possible structures related with stimulus. In order to observe inside the sensila and to avoid the auto-fluorescence from the chitinous exoskeleton, the samples were treated with chitinase. The fluorescent lectins labeled sugar residues located in distinct regions of the sensila differentiating the two sandfly species. The fluorescent phaloidin revealed the cytoskeleton of the sensila and the associated-cells present in its insertion region on the antenna. The fluorescein vital stain and the PKH-26 marker allowed us to detail the morphology and the cell composition of the organ. In conclusion, the use of the confocal laser microscopy associated with fluorescent markers allowed us to make important observations of the chaetic sensila. The knowledge of the sensorial organs related with the olfactory capacity of vectors can contribute to design future strategies for controlling disease transmission. Supported by: CNPq, Fapemig, Pronex and Fiocruz.

VE31 - COMPARATIVE STUDY OF THE ANTENNAE SENSILA OF THE THREE POPULATIONS OF Lutzomyia longipalpis FEMALES, VECTOR OF VISCERAL LEISHMANIASIS.

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Lutzomyia longipalpis is the main vector of Leishmania chagasi, the causative agent of American visceral leishmaniasis. Morphological studies, isoenzyme, pherohormones analysis and molecular markers suggest that L. longipalpis is a complex of species. This fact induced us to try to understand the biology and the morphology of the sensorial organs located in the antennae, which can be involved in recognizing stimulus from the environment. In this study, we statistically analyzed and compared the morphology of the sensila from three populations geographically distant of the L. longipalpis (Cavunge/BA, Jequié/BA e de São Luís/MA). We used the scanning electron microscopy to visualize, classify and count the numbers of sensila in each female sandfly antennae. Our results pointed out that all the sandflies have one pair of long and thin antenna composed by sixteen segments: scape, pedicel and fourteen flagelomeres covered by fine hair-like structures called microtrichias. We morphologically identified five types of sensila: chaetic, basiconic, coeloconic (with two subtypes - "hands on pray"and sulcada), trichoid (with three subtypes - small, pointed-tip and blunt-tip) and squamiform. The quantification and distribution of each sensilae type on all antennae segments were done. The Student test (T test) demonstrated significant differences in the number and location of the three studied populations. These observations can help future taxonomic and philogenetic study with the objective of clarifying the evolutionary aspects of L. longipalpis. Supported by: CNPq, Fapemig, Pronex and Fiocruz.

VE32 - Comparative study of the antennae sensila of *Aedes aegypti*, *Aedes albopictus* and *Aedes fluviatilis* by SCANNING ELETRON MICROSCOPY.

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The *Aedes aegypti* is the main vector of dengue and yellow fever viruses in the Americas. The *Aedes albopictus*, which recently entered in Brazil, is a potential vector of dengue since it has an important role in spreading the disease in Asia. These two mosquito vectors have vectorial competence for the four sorotypes of dengue virus. The *Aedes fluviatilis* is an excellent experimental vector of avian malaria mainly in countries where the *A. aegypti* is capable of transmitting human diseases. These mosquitoes are anthropophilic being found in distinct urban environments with different habitats. Mosquitoes in general have developed numerous specializations to locate blood source, for example the sensila in the antennae, which allows them to monitor specific stimulus from the environment. Sensila are sensorial organs that are located on the insect's body parts and are capable to detect odors, temperature, humidity, mechanical stimulus and infrared radiation. The objective of this study is to comparatively analyze the sensila present in the antenna of mosquito females of the three species by scanning electron microscopy. In small magnification of the heads, the three mosquito species presented similar aspects with one par of antenna covered by fine hair-like structures, the microtrichias. Each antennae is composed by scape, globular pedicel and thirteen flagelomeres. Large magnification views revealed three types of sensila: squamiform, campaniform and trichoid (with the subtypes: small, fine-tip and blunt-tip with veins). The amount and the distribution of these sensila in the flagelomeres varied according to the mosquito species. In conclusion, our ultra-structural observation of the antennae sensila of these mosquito vectors can be considered important findings for taxonomic studies and also can help to design strategies for mosquito control using semiochemicals. Supported by: CNPq, Fapemig, Pronex and Fiocruz.

VE33 - Aspects of the interaction between *Leishmania chagasi* and *Lutzomyia longipalpis*, the vector of visceral leishmaniasis

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Lutzomyia longipalpis is the main vector of *Leishmania chagasi*, the causative agent of American Visceral Leishmaniasis. The development of *Leishmania* in the a sandfly vector is a complex process in which the parasites undergo changes from a non infective form (procyclic promastigote) to an infective form (metacyclic promastigote). Some investigations have already described detailed molecular and structural aspects of this interaction, mainly in Old Word species. The objective of this work was to characterize *L. chagasi* development in *L. longipalpis*. Female sandflies were infected using glass feeder with blood containing 10^7 promastigotes. Sandfly midguts were dissected and observed until the 6^{th} d after the infective blood meal. The percentage of fed sandflies was 38.3% (36/94). The mean proportion of infected sandflies was 94.4% (34/36) and this value decreased on the 6^{th} d of infection. The highest parasite density occurred at day 4, gradually decreasing until 6^{th} day. All the promastigote forms were found in the sandfly midguts and their number

varied throughout the infective evolution. In the beginning of the infection, the procyclic and the nectomonad were the predominant forms in the midguts (42% and 58% respectively) diminishing at the 6^{th} day (8% and 9% respectively). The haptomonad had a continuous growth until reach 73% in the last day of our experiment. The nectomonads were observed since the 2^{th} d of infection with a gradual decrease until the 6^{th} day. Paramastigote forms were observed in small numbers. The metacyclic forms appeared at the 3^{th} d with growth peak at day 5 of infection (7%). Further experiments are being done for better understanding this process of *L longipalpis*. Studies on Leishmania-vector interactions aimed to further understand the many processes engaged in the transmission of the parasite, which are necessary to elucidate aspects of leishmaniasis epidemiology. Supported by FIOCRUZ/IRR, PAPES IV and AMSURD

VE34 - Glycoconjugate distribution in *Lutzomyia longipalpis* midgut cells analyzed by lectin cytochemistry

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Lutzomyia longipalpis sandfly is the main vector of *Leishmania chagasi*, agent of visceral leishmaniasis in South America. The parasite life cycle starts in the sandfly with ingestion of an infective blood meal. The midgut epithelium synthesizes a thick bag-like structure that surrounds the blood meal, named peritrophic matrix (PM). One of the major roles of PM is to protect the sandfly against ingested pathogens by providing a defensive barrier. The PM is composed by chitin (N-acetylglucosamine) and others sugar polymers. A cytochemical lectin-binding study was performed to investigate the distribution of the glycoconjugates in epithelial midgut cells comparing both male and female sandflies. The fluorescent lectins used in this work were RCA, BS-1, LPA, ConA and WGA. The samples were analyzed in a Confocal Laser Microscope. The control samples showed a basal autofluorescence characteristic of the insect tissues. The RCA and BS-1 labeled indistinctly the midguts, both in male and female sandflies. The RCA marked the periphery of the cells and the BS-1 showed a perinuclear reaction. The LPA displayed a weak reaction in the female sandflies, but showed many fluorescent pigments in the male sandflies. The ConA labeled distinctly the midguts of the analyzed groups. Female sandflies showed strong reactions in some cells and the muscle fibers were negatives. However, male sandflies showed several fluorescent pigments in the epithelial cells and strong labeling in the muscular fibers. In both male and female sandflies, the lectin WGA displayed a ubiquitous distribution of the epithelial midgut cells and the muscle fibers. These results showed that the sugar composition of the midgut cells varied between male and female sandflies. These differences could de due to the exclusive sugar diet of the male sandfly, in contrast of the haematophagous and sugar diet of the female. Supported by Fiocruz, Fapemig, CNPq, Pronex and CAPES

VE35 - The study of the *Aedes aegypti* oenocytes

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The insect oenocytes are ectodermic cells that are involved with neurogenesis, with nutrients transport and metabolism, and with insecticide resistance. In general they are physically associated with the trophocytes in the Diptera fat body. The objective of the present work was to morphologically characterize the Aedes aegypti pupae oenocytes in primary culture. Oenocytes showed different FITC-lectin staining patterns, depending on the lectin. For example, for LCA, oenocytes were strongly stained, showing a cell surfaces rich of glucose resides, while for ConA, oenocytes were weakly stained, but the basal lamina was positive for this lectin, suggesting the presence of the á-mannose and α-galactose resides in extracellular matrix. The oenocytes were positive for UEA and for BSA, suggesting the presence of α-L-fucose and á-galactose on the cell surface and α-gal-N-acetilglucosamine residues in the intercellular space. The semi-thin sections showed that the oenocytes are oval-shaped cells with a central nucleus and several unstained cytoplasm structures. Under transmission electron microscope, these structures corresponded to the cell canalliculi system. This system is formed by cell membrane infoldings that invaginated deeply from the cell surface to the nucleus vicinity. The canalliculi ramified is seen like a complex reticular system that probably corresponds to the smooth endoplasmic reticulum, resembling steroidogenic cells. The well developed reticular system suggested that oenocytes are cells committed to the synthesis and releasing of lipid. In addition, we found cell-adhesion specializations that corresponded to the fillopodia. The oenocyte nucleus has a well developed nucleolus and a ribosome-rich cytoplasm. Also, polyribosomes were found surrounding the nucleus envelope, suggesting that in these cells, the protein synthesis is intense. Considering that mosquito oenocytes are poorly understood, ours results contributed to the understanding of this cell function. Supported by: CNPq, Fapemig, Pronex and Fiocruz

VE36 - Morphologic study of the salivary gland of *Aedes aegypti* (Linnaues, 1762) and the salivary gland infected by the dengue virus (denv-2)

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The principal urban vector of dengue virus is the *A. aegypti*, a highly domesticated mosquito that has adapted to humans. The dengue virus serological group of the family Flaviviridae, genus Flavivirus, consists of four related virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). The salivary gland of the mosquitoes and their secretions play an important role in the hematophagism of the insects and consequently in pathogen transmission, as for example the dengue virus. Conventional histology, microscopy of transmission, scanning and laser confocal associates with fluorescent markings and imunocitoquimic were done to study the organization and the structure of the cells of the salivary gland. The objective of this work was to study the morphological aspects of salivary gland and the distribution of carbohydrate epitopes in the salivary gland of *A. aegypti*. Also, oral and intrathoracic susceptibility of colonized mosquitoes to dengue virus was evaluated. The gland is paired structures, where each lobe consists of three lobes. They have in sac shape, composed by unique layer of cells, with basal and apical regions. Two secretory portions called proximal and distal, joined by a poorly defined transition region, compose each lateral lobe; the central lobe is formed by a single secretory portion. A non-secretory portion is seen at the proximal region of the medial lobe. Variations in the intensity and binding of lectins demonstrated the presence of diversity in the specific carbohydrate epitopes on the basal lamina, salivary duct and in the region of contact between the cells with secretory cavity. Immunolabeling for dengue virus showed that the salivary gland was infected 15 days after artificial infection. The RT-PCR reactions showed that the mosquitoes are susceptible to virus dengue infection, confirmed through the detention of viral RNA in the head, the body and the fat body, 7 days after infection.

VE37 - Plasmodium Gallinaceum OOCYST FORMATION IN THE VECTOR MIDGUT: MODIFICATIONS IN THE OOCYST WALL AND IN THE VECTOR MUSCLE NETWORK.

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Mosquito infection with *P. gallinaceum* provides a good model for analyzing the interaction process. After the infective blood meal with *Plasmodium*, the parasites are stored in the mosquito midgut. In susceptible vector, the midgut invasion by the parasites with subsequent oocyst formation is a crucial event in the infection process. This study has two goals: (a) analyze the oocyst formation and (b) verify associated changes in the midgut muscle layer of the vector. Infected midguts were analyzed by laser confocal microscopy (LCM) and scanning electron microscopy (SEM). For the LCM, the midguts were labeled with Phalloidin/FITC (actin marker) and with To-pro 3 (DNA-nuclear marker). Also, we used fluorescent lectins (Con A and PNA) for labeling sugar residues in the oocyst wall. SEM was used to observe the topography of the oocyst development and possible alteration in the midgut muscle layer. The Phalloidin/FITC labeled in green color the cytoskeleton of the midgut cells including the muscle fibers, showing the presence of actin filaments. The ooscysts were well stained in red by To-pro 3 contrasting with the green muscle fibers. Our results demonstrated anatomical modification of the muscle fibers in the organization of the muscle network. The muscle fibers were disturbed by the oocyst development. The SEM confirmed details of these structural modifications and also allowed us to observe the complete oocyst formation until the release of sporozoites. In addition, the fluorescent lectins showed the presence of sugar residues in the oocysts wall with changes according to their formation. In conclusion, this study showed that the midgut muscle layer changes due to the oocyst development. Also, we showed that the oocyst wall also has morphological changes associated with exposition of sugar residues. Financial support: Fapemig, Pronex, CNPq and Fiocruz.

VE38 - The morphology of the *Aedes fluviatilis* fat body and the DNA fragmentation caused by the *Plasmodium gallinaceum* infection.

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The insect's fat body is the organ responsible for the intermediary metabolism and nutrients supply. It is known that during its life cycle, parasites can reduce the insect fitness compromising physiological functions including their development and reproduction. The objective of this work was to investigate the morphology of the *A. fluviatilis* fat body and the effect of the *P. gallinaceum* infection in the organ. Under scanning electron microscope, the fat body is formed by lobes that are projected from body wall to the hemocele. These lobes are formed by clusters of fat body cells that are packaged together by a basal lamina. The histological sections showed that the insect fat body is formed by a multicellular layer located beneath the integument. Two cell types exist in the fat body: trophocytes and oenocytes. The trophocytes are storage-like cells that have the cytoplasm filled by lipid droplets and protein granules. The oenocytes are ectodermic cells. They are located mainly in the fat body periphery and their cytoplasms are uniformly stained for proteins as

seen by Gomori's stain. Other authors have demonstrated that some parasites can affect the fat body cells depleting the cytoplasm storage and leading to cell apoptosis. In order to understand the effect of the infection in the fat body, we analyzed seven days after an infective blood meal, the DNA fragmentation. The fat body had several nuclei stained by TUNEL reaction, mainly near the ovaries, while the control had a few stained nuclei. This data suggested that the *Plasmodium* infection increased the DNA fragmentation in the mosquito fat body. It is known that apoptosis increases in parasitized insects. However, whether or not the DNA fragmentation is a result of the apoptosis, still needs to be investigated in the *Aedes-Plasmodium* system.

VE39 - Inferring through Cyt b gene variation the *Triatoma brasiliensis* Neiva 1911 (Hemiptera: Reduviidae: Triatominae) Genetic Structure, Domiciliary Invasion and Infestation in the State of Paraíba, Brazil

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The difficulty of controlling *Triatoma brasiliensis* has been attributed to its capacity to occupy the wild, peridomiciliary, and domiciliary environments. One of the key questions of population genetic studies on triatomines is whether ecotopic populations distant from each other would exchange migrants more often than physically close ones differing by ecotope. Relative levels of dispersal within and among spatially or ecologically structured groups of populations can be compared using neutral genetic markers, and components of the landscape that limit dispersal can be identified using population genetics inferences. The genetic structure of *T. brasiliensis* was analyzed by mitochondrial gene variation between geographic location and ecotopes in a short and long period after domiciliary insecticide treatment in the state of Paraíba, Brazil. Four different localities (ranging from 16 to 40 km apart) were sampled. Analysis of molecular variance (AMOVA) showed that grouping populations according to the geographic location resulted in a higher variance within localities than among them. A similar pattern was obtained if we grouped populations by ecotope (FSC = 0.15 and 0.17, FCT = 0.07 and 0.04, respectively). The percentage of variation was increased between groups and reduced between sites within groups (FSC = 0.08, FCT = 0.16) by grouping (i) the domiciliary populations from each village, (ii) all ruderal populations, and (iii) the sylvatic one. The data obtained showed that within the area studied *T. brasiliensis* is genetically structured both ecological and at smaller geographical scales. The pattern of recolonization was analyzed by comparing Cyt B gene variation of wild and domiciliary populations and by comparing populations from a short and long time after insecticide spraying. Reinfestations after insecticide treatment were also composed by distinct populations. These results indicate that the variable population sources for domiciliary infestations should be considered for control measures. Financial Support: CNPq and CAPES.

VE40 - TWO DIFFERENT ISOFORMS OF CATHEPSIN-L ENCODING GENES IN THE DIGESTIVE TRACT OF *TRIATOMA BRASILIENSIS* (HEMIPTERA: REDUVIIDAE)

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The protein digestion in insects is a complex process in which several enzymes with proteolytic properties are involved. The majority of blood sucking insects use serine proteases (e.g. trypsins and chymotrypsins) as major digestive enzymes, while Hemiptera use cysteine proteinases, e.g. cathepsin B, D and L. Cathepsins are intracellular enzymes, which usually occurs in lysosomes in which they find the essential acidic conditions for the hydrolysis of peptide bonds. In Hemiptera, which possess an acidic environment in the small intestine, they are secreted into the midgut lumen for hemoglobin digestion. To date, cathepsin B (EC 3.4.22.1) is the best studied cysteine proteinase and was isolated from several species. Considering triatomines, cathepsin encoding genes from two species are described: cathepsins B and L from *Rhodnius prolixus* and *Triatoma infestans*. In insects, which can be infected by pathogens, such as triatomine species with *Trypanosoma cruzi*, the proteolytic activities might also play a role in the establishment and maintenance of the parasite in the gut. The aim of the present study was to isolate and characterize cathepsin encoding genes from the intestinal tract of *Triatoma brasiliensis*, which is an important *T. cruzi* vector in the north-eastern region of Brazil. During the experimental procedures, two isoforms of cathepsin L encoding genes (*tbcat-1* and *tbcat-2*) were identified and both fragments are constituted of 497 bp. Future experiments to obtain full sequences and investigate the expression pattern of these genes will be done. *Key words*: Cathepsins, digestive tract, *Triatoma brasiliensis*, digestive enzymes. Financial Support: CNPq (472276/2006-9), FAPERJ (E-26/170.122/2004) and FIOCRUZ.

VE41 - GENETIC DIFFERENTIATION BETWEEN BRAZILIAN POPULATIONS OF *ANOPHELES CRUZII* USING CLOCK GENES AS MOLECULAR MARKERS

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Anopheles cruzii (Diptera:Culidae) is a malaria vector belonging to the *Kerteszia* subgenus, being responsible for endemic disease in southern Brazil between 1930 and 1960. Nowadays, it is responsible for some cases of the disease in the southeastern coast of Brazil. The *An. Cruzii* taxonomic status is unclear. The analysis of the banding pattern of the X chromosome inversions frequencies of populations from Southeastern and Southern Brazil revealed three sibling species. Another study using isoenzymes reported two groups genetically isolated, the first one from Bahia State and the other from Southeastern and Southern Brazil States. In the present study, the *timeless* gene, a locus involved in the control of circadian rhythms, was used as a molecular marker to analyze the genetic differentiation between five *An. cruzii* populations from Brazil. Our results show that the mosquitoes from Bahia State (Northeast Brazil) constitute a different group from the other four populations from South and Southeast Brazil. These results strongly suggest that *An. Cruzii* is a complex of at least two cryptic sibling species. Besides the *timeless* gene we are also using different clock genes to analyze the differentiation between the populations from Bahia and Santa Catarina (South Brazil) States.

VE42 - Blood-feeding- and infection-induced differential gene expression in the Brazilian malaria vector *Anopheles aquasalis*.

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Malaria still affects 300 million people worldwide every year, causing 1.5-2.7 million deaths. In Brazil there are 450.000 cases per year, 99.7% of which occur in the Amazon region. Molecules involved in the interaction between mosquito vectors and plasmodia have been the focus of studies aiming at novel malaria control strategies. However, almost all such studies are based on Old World anopheline species. Thus, our main goal is to analyze the effects of blood-feeding and infection with *Plasmodium vivax* on gene expression induction in the Brazilian malaria vector, *Anopheles aquasalis*. For that, *A. aquasalis* were fed with blood of healthy volunteers and also on patients infected with *P. vivax*. The cDNAs obtained were subtracted to obtain expressions libraries specific for the different experimental conditions. These differentially expressed cDNAs were cloned, sequenced and analyzed with appropriate bioinformatics tools. After the subtraction analysis, important differences in gene expression were detected in *A. aquasalis* after feeding and infection for the two established times (two and 24 hours). For example, expression of fibrinogen cDNA was observed only in the infected insects, whereas digestive enzymes cDNAs′ (chymotrypsin, serine proteases, carboxypeptidases) were detected solely in the blood-fed insects. In parallel, seven genes involved in the cellular detoxification and immune response were PCR-amplified using degenerate oligonucleotides. One detoxification-related gene (dismutase superoxide) and three immune system genes (STAT, PIAS and NOS) were successfully amplified, cloned, and sequenced. The expression of these genes is being evaluated at different times of the *Plasmodium* life cycle inside the insect vector. These results indicate that genes are differentially expressed during *A. aquasalis* feeding and infection with *P. vivax*, generating important information on molecules possibly involved in the interaction process of this Brazilian vector with the malaria parasite.

VE43 - The *Rhodnius prolixus* Genome Project and the genetic typing of world colonies

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Rhodnius prolixus is one of the most important primary vectors of human Chagas disease in Latin America. As a consequence of its epidemiological relevance (together with other reasons such as its smaller genome size when compared to that of *Triatoma infestans*, for example), this species has been elected as the first hemimetabolous insect to have its genome sequenced. A crucial step in any genome project is the choice of strain to be used as source of DNA. As the morphology of *R. prolixus* is virtually identical to that of the members of the closely related *R. robustus* cryptic species complex (which includes secondary vectors), it was necessary to genetically confirm the identity of candidate laboratory colonies of *R. prolixus*. A 682 bp fragment of the mitochondrial cytochrome *b* gene and the 912 bp nuclear ITS-1 and ITS-2 ribosomal regions were sequenced for 52 *prolixus* and *robustus* field-collected specimens (representing 10 populations) providing high discriminatory power and congruent topology and, thus, were chosen as genetic markers. During an initial screening, Brazilian *R. prolixus* colonies revealed signs of hybridization and introgression with *R. robustus* (*i.e.* insects presenting *prolixus* ITS but *robustus* mitochondria). Thus, we decided to investigate how widespread was

the phenomenon by genotyping 13 *R. prolixus* colonies from around the world (Argentina, Brazil, Canada, Colombia, Guatemala, Switzerland, United States, and Venezuela). Approximately 20 insects were sequenced per colony for the two genes (totaling 584 sequenced samples). Only four colonies were pure *prolixus i.e.* presented congruence between both markers. Curiously, all hybrid colonies (which included one descended from Wigglesworth`s colony) were introgressed with the same *robustus* IV haplotype. We recommended the Guatemalan and CDC colonies for the "survey sequencing' step of the Genome Project destined to determining average heterozigosity levels, and the CDC colony was elected for having the lowest heterozigosity.

VE44 - Transcriptome of *Triatoma infestans* Salivary Glands

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Triatoma infestans is one of the most important vectors of Chagas Disease in Latin America, feeding on vertebrate blood in all life stages. Hematophagous insects salivary glands produce potent pharmacological compounds that counteract host hemostasis, including anti-clotting, antiplatelet, and vasodilatory molecules. To obtain a further insight into the salivary biochemical and pharmacological complexity of this insect, a cDNA library was randomly sequenced. Also, salivary proteins were submitted to 2D gel electrophoresis followed by MS analysis. We present the analysis of a set of 1,534 salivary gland cDNA sequences, 645 of which coding for proteins of a putative secretory nature. Most salivary proteins described as lipocalins - 55% of the cDNA library - matched peptides sequences obtained from proteomic results. We expect this work will contribute with new salivary transcripts that could help the understanding of the role of salivary molecules in host/vector interactions and the discovery of novel pharmacologic agents. Supported by CNPq and CAPES.

VE45 - EST sequencing of *Lutzomyia longipalpis* cDNA libraries constructed from leishmania infected and non-infected gut

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Leishmaniasis is a serious public health problem world-wide, with a large distribution of the disease. According to WHO, the endemic regions for leishmaniasis, as well as case numbers, have increased. In Brazil, visceral leishmaniasis is caused by *Leishmania chagasi*, transmitted mostly by *Lutzomyia longipalpis*. Although these insects are important vectors for leishmaniasis and other diseases, there is little information available related to parasite-vector relationship and innate immunity. In this context, this work aims at performing the *L. longipalpis* gut transcriptome, by sequencing ESTs from expression libraries constructed in Lambda Zap. These libraries were generated from RNA extracted from *L. longipalpis* gut 6 and 72 hours after blood meal, and 72 hours after artificial infection with *L. chagasi*. A total of 3711 clones were sequenced, with 2520 sequences showing high quality, corresponding to 832.5 Kb. In the infected library 491 singlets and 179 clusters were found. In the 72 hours blood fed library, 473 singlets and 83 clusters were found. In the 6 hours blood fed library, 327 singlets and 68 clusters were found. After clusterization and annotation, 1504 sequences were identified. The annotation process revealed several genes related with peritrophic matrix physiology, stress and blood meal digestion, such as mucin, trypsin, trypsin inhibitor, chymotrypsin, ferritin, among others. Sequences related to processes like apoptosis, defense, oxidation, signal transduction and cell adhesion were found mostly in the infected library. On the other hand, sequences related to blood digestion were predominantly identified in the 6 hours blood fed library. Twenty one percent of the sequences (317) had no Blast hit against GenBank, Pfam, Conserved Domain Database (CDD) and Refseq. These EST data will be useful for understanding this neglected vector and for the dentification of potential targets in the vector-parasite relationship.