Quimioterapia-Chemotherapy

QT01 - THE CRUDE ETHANOLIC EXTRACT, THE LIGNOIDS FRACTION AND THE YANGAMBIN OBTAINED FROM Ocotea duckei Vattimo (LAURACEAE) PRESENT ANTILEISHMANIAL ACTIVITY

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Leishmaniasis constitutes a complex group of infective parasitic diseases caused by protozoans of the genus Leishmania. The chemotherapy of leishmaniasis is based on the administration of pentavalent antimonials (Glucantime and Pentostan), pentamidine or amphotericin. These agents present a high toxicity in humans and augmentation of parasites resistance to the drugs. These drawbacks show that new therapeutic agents for treating leishmaniasis are urgently necessary. Several compounds isolated from plants have presented as antileishmanial activity. It was aimed in the present work to identify compounds that have therapeutic potential against leishmaniasis, investigating the activity of crude ethanolic extract (CEE), lignoids fraction (LIGF) and the purified compound yangambin (Yg) (obtained from Ocotea duckei, Lauraceae) on the promastigote forms of Leishmania (L.) chagasi and Leishmania (L.) amazonensis cultivated in Schneider medium, supplemented with 10 percent of foetal calf serum. The CEE, LIGF and the Yg showed IC50 of 512microgram/mL, 26.52microgram/mL e 49microgram/mL respectively on Leishmania (L.) chagasi, while on promastigote forms of Leishmania (L.) amazonensis the IC50 was 143.74microgram/mL, 48microgram/mL e 65.13microgram/mL respectively. The inhibition of promastigotes growth by lignoids fraction can be, in part, due to yangambin, since this is the main lignan found in that fraction. The antileishmanial activity of the yangambin lignan has not yet been reported, but other pharmacological activities have been reported with respect to its anticonvulsive, analgesic and antiallergic effects. The derivates obtained from Ocotea duckei show activity against promastigote forms of Leishmania showing therapeutic potencial to the treatment of the leishmaniasis. Citotoxicity assay and ultrastructural analysis have been investigate to characterize their activities.

QT02 - THE SYNTHETIC COMPOUND 2-methylene-3-(4-brominephenyl)-propanenitrile PRESENT HIGH ACTIVITY AGAINST Leishmania (L.) chagasi

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Leishmaniasis constitutes a complex group of infective parasitic diseases caused by protozoans of the genus Leishmania. The chemotherapy of leishmaniasis is based on the administration of pentavalent antimonials (Glucantime and Pentostan), pentamidine or amphotericin. These agents present a high toxicity in humans and augmentation of parasites resistance to the drugs. These drawbacks show that new therapeutic agents for treating leishmaniasis are urgently necessary. Substances obtained from chemistry synthesis have already been identified as antileishmanial activity. In the present work we investigate the activity of the 2-methylene-3-(4-brominephenyl)-propanenitrile (1), a substance chemically synthesised, on the promastigote forms of Leishmania (L.) chagasi cultivated in Schneider medium, supplemented with 10 percent of foetal calf serum. The compound 1 was obtained through the Baylis-Hillman reaction. This reaction has been employed on synthesis of bioactive substances. The compound 1 showed IC50 of 6microgram/mL on Leishmania (L.) chagasi. These data show that drug exhibit clearly a high activity against L. chagasi suggest therapeutic potential these compound to the treatment of leishmaniasis. Assays using the amastigote form of Leishmania (L.) chagasi have been investigate.

QT03 - The antiestrogen tamoxifen induces alkalinization of parasitophorous vacuoles harbouring Leishmania amazonensis - Implications for parasite survival

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Parasites from the genus *Leishmania* are ethiological agents of leishmaniasis. The chemotherapy of this disease relies mainly on the administration of pentavalent antimonials that are toxic, poorly tolerated and are becoming ineffective due to increase in resistant *Leishmania* strains. This study reports an investigation on the activity of tamoxifen, an antiestrogen widely used in the treatment of breast can-

cer, against *Leishmania amazonensis*. Tamoxifen killed promastigotes and amastigotes with in vitro 50 % inhibitory concentrations of 16.4± 0.2 μM and 11.1± 0.2 μM, respectively. We also analyzed pH changes inside parasitophorous vacuoles of peritoneal macrophages infected with *L. amazonensis* amastigotes, monitoring the compartmentalization of SNAFL-calcein-AM probes. Our data show that 10 μM tamoxifen induces a rapid alkalinization of the vacuolar environment from pH values of approximately 4.0 to 8.0. In parallel, we also provide evidence that tamoxifen is more effective in vitro against amastigotes at pH 7.5 when compared to cultures at pH 4.5. Bearing in mind that amastigotes are unable to survive outside the parasitophorous vacuole of macrophages in their mammalian hosts, it seems to be a valuable approach to interfere at amastigotes' optimal settings. Our results thus suggest that tamoxifen modulates intravacuolar pH and may affect the survival of parasites by direct and indirect pathways, once it inhibits parasite growth in vitro, with higher effectiveness at pH 7.5, and is also capable of changing the parasitophorous vacuoles optimal pH where parasites live and multiply in host cells. Supported by FAPESP.

QT04 - The inhibitory effects of the calpain inhibitor MDL28170 against *Leishmania amazonensis*

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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 and morphology of *Leishmania amazonensis*. Briefly, promastigotes were resuspended in fresh medium to a final concentration of 1.0 x 10^6 viable promastigotes per milliliter. The inhibitor was added to the culture at final concentrations of 15, 20, 25 and 30 μM. The calpain inhibitor at 30 μM promoted a powerful reduction on the cellular growth rate by approximately 38, 90, 94 and 95% after 24, 48, 72 and 96 h, respectively. The lowest concentrations of the drug (15 and 20 μM) presented significant inhibitory effects only after 72-96 h of growth. Corroborating these results, optical microscopy observations showed a massive deterioration of promastigote cells after treatment of the parasites with 30 μM MDL 28170 for 48 h. Based on the effects of MDL28170 on the growth rate and morphology of *L. amazonensis*, we aimed to detect calpain homologues in this protozoan by immunoblot assays using different anti-calpain antibodies. The antibodies against *Drosophila melanogaster* calpain (Dm-calpain) strongly recognized a polypeptide band migrating at approximately 80 kDa. No common epitopes were found between mammalian calpains and *L. amazonensis* polypeptides. The calpain-like molecule was detected on the cell surface of *L. amazonensis*, as demonstrated by flow cytometry and fluorescence microscopy analyses using the anti-Dm-calpain antibody. The immunofluorescence image showed a labeling throughout the cell surface, including the flagellum.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

QT05 - NATURAL PRODUCTS AS NEW ANTILEISHMANIALS: ACTIVITY OF BRAZILIAN PLANT EXTRACTS AGAINST LEISHMANIA (L.) CHAGASI AND LEISHMANIA (L.) AMAZONENSIS

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Leishmaniasis is an endemic disease found mainly in tropical and subtropical countries and afflicts the poorest population of the world. It is estimated 12 million cases with 2 million new cases/year. Visceral Leishmaniasis, which contributes to 500.000 cases, is a progressive and fatal disease if not adequately treated. Cutaneous Leishmaniasis contributes from a single cutaneous ulceration to a disfiguring disease. The toxic antimonials are still the main choice for the treatment in Brazil, despite of resistant cases related in many countries. Brazilian flora represents an important tool for Drug Discovery studies, supplying new organic compounds which could be used as prototypes in the development of new chemotherapeutics against parasitic diseases. In this work, we have tested in vitro 16 Brazilian methanol plant extracts against *Leishmania L.chagasi* and *Leishmania L.amazonensis* to the highest concentration of 500μg/mL. Five methanol extracts presented antileishmanial activity against *L.chagasi* promastigotes: *Plectranthus amboinicus* with an EC_{50} of 45.14μg/mL, *Aristolochia cymbifera* – EC_{50} of 89.17μg/mL, *Plectranthus barbatus* –EC_{50} of 54.46 μg/mL, *Lippia alba* – EC_{50} of 62.67 μg/mL and *Hydrocotyle banariensis* –EC_{50} of 235.0μg/mL. From these five extracts, four presented activity below 100μg/mL, representing an important data if one consider future isolations of active substances. *L. amazonensis* promastigotes were more resistant than *L. chagasi* against *Hydrocotyle banariensis* (EC_{50} of 302.5μg/mL). Some other extracts showed activity against *L. amazonensis* but were not effective against *L. chagasi*promastigotes: *Bacaris trimera* with an EC_{50} of 374.3 μg/mL, *Cymbopogon citratus* (EC_{50} of 113.1μg/mL) and *Plectranthus grudis* (EC_{50} of 188. 0 μg/mL). Our preliminary data shows the importance of natural secondary metabolites derived from plants for Drug Discovery Studies, which could be helpful in design of new chemotherapeutics for Leishmaniasis. This work was supported by Instituto

Adolfo Lutz and Instituto Plantarum de Estudos da Flora.

QT06 - Activity of the tellurium compound RT01 in the experimental model of *Leishmania amazonensis* infection

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Leishmania amazonensis causes human diseases that range from self-healing cutaneous lesions to difusion cutaneous leishmaniasis. Chemotherapy tends to be difficult, often requiring prolongued administration of toxic drugs such as pentavalent antimonials. In the present study we evaluate the *in vitro* and *in vivo* effects of RT01 in *L. amazonensis* infection. The RT01 compound ($C_{13}H_{22}N^{+}{\cdot}C_3H_3Cl_4OTe^{-}$) is a synthetic compound belonging to the organotellurium family. Tellurium-based compounds has immunomodulatory properties and anti-intra-erythrocytic parasite *Babesia rodhaini* activity. We found that RT01 caused an inhibition of the promastigote growth (80-100%) after 48 h of treatment with 3, 5 10 and 15 μg/ml. The *Leishmania* differentiation was also drug sensitive: there was a 100% reduction in amastigote to promastigote differentiation at 1 μg/ml RT01. BALB/c mice were infected with 10^5 amastigotes of *L. amazonensis*, and at days 1, 15 and 30 post-infection were treated intraperitonealy with RT01 (72, 108 and 144 μg/animal). Lesion size and lesion parasite burden were weekly monitored by 40 days. The data obtained in this study, demonstrated that, when compared to controls, mice treated with RT01 (72 μg/animal) had smaller lesions (50%) and lower parasite burden at 5 weeks after infection. The RT01 showed no toxicity on mice. Our results suggest that tellurium based compounds have promising antileishmanial potential. Supported by FAPESP,CAPES, CNPq and SAE.

QT07 - Antileishmanial activity of dichloromethane and hexanic fractions obtained from *Allamanda schottii*

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Leishmaniasis is a group of diseases affecting millions of people around the world. Its treatment generates a series of side effects, as it makes use of very toxic drugs, and has not proved sufficiently effective because of the emergence of *Leishmania* parasites resistant to them. Therefore, the search for alternative treatments is necessary. With this purpose, this study was carried out to evaluate the antileishmanial activity of the dichloromethane and hexanic fractions of *Allamanda schottii* against promastigotes of *Leishmania amazonensis* and *Leishmania chagasi*. Dichloromethane fraction showed significant activity against *L. amazonensis* and *L. chagasi*, with IC50 of 60.9 μg/mL and 72.41 μg/mL, respectively; regarding the hexanic fraction, the corresponding figures were 123.35 μg/mL and 224.88 μg/mL. An investigation of the cellular viability of *L. amazonensis* and *L. chagasi* cultures in the absence and in the presence of *A. schottii* fractions was conducted, with the use of 0.01% eritrosine, which permits identifying living and dead cells. A large amount of dead cells in the presence of the IC50 of the fractions was observed, which demonstrates that these fractions cause strong toxic effects on *Leishmania* parasites. This led to testing the toxicity of the fractions towards mammal cells, with a view to the therapeutic use of the finding. Both *A. schottii* fractions showed a significant toxicity to peritoneal mouse cells, comparable to their antileishmanial activity, which suggests that they may cause side effects on mammals. Thus, new experiments with compounds isolated from the fractions need to be done to identify which of them are responsible for the antileishmanial activity observed and to see if they may cause toxic effects on mammal cells.

QT08 - New synthetic naphthoquinones that are highly inhibitory for Leishmania amazonensis.

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Quinones have been largely studied for antitumor, molluscicidal, antiparasitic, antiinflammatory, antifungic, antimicrobial, and trypanocidal activities. In this work, we demonstrate the antileishmanial activity of new synthetic naphthoquinones LQB 17; LQB 18; LQB 19; LQB 20; LQB 22; LQB 94; LQB 95; LQB 114 and LQB 118 and used lapachol, α-lapachone and β-lapachone as control drugs. Promastigotes of Leishmania amazonensis were cultivated for 72h with various concentrations of the quinones in 199 medium supplemented with 10% fetal bovine serum. The β-lapachone presented strong antipromastigote activity, with IC50 at 2,5 uM. Quinones LQB 17, LQB 18, LQB 19, LQB 22, LQB 95 and LQB 118 were also active, with IC 50 in the range of 5 to 10 uM. The remaining LQB 20, LQB 94 and LQB 114 presented IC 50 higher than 20 uM and their study was discontinued. The promastigote-active quinones were tested on intracellular amastigotes. Antiamastigote activity was evaluated by incubating infected peritoneal macrophages with the drugs for 72h. When tested at 3,75 uM, LQB 22 and β-lapachone inhibited more than 80% of parasite growth; α-lapachone, LQB 17, LQB 18, and lapachol inhibited 70% and LQB 20, LQB 95, and LQB 114 inhibited 60%. Pentostam at 50ug/ml inhibited 82%, less active than β-lapachone. These results show for the first time the selective antileishmanial activity of the LQB naphtoquinones series, and indicate LQB 22 and β-lapachone as the best prototypes for in vivo evaluation. CNPq.

QT09 - ANTILEISHMANIAL CHALCONES WITH INCREASED IN VITRO ACTIVITY AGAINST AFRICAN TRYPANOSOSMES.

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At present, the chemotherapy of trypanosomiasis and leishmaniasis depends on a relatively small number of synthetic drugs and is associated with significant toxicity. So, new active drugs with high selectivity are desired to ameliorate the treatment of these protozoan diseases. We showed previously the therapeutic effectiveness of the natural chalcone 2'-6'-dihydroxy-4'-methoxychalcone (DMC) and its synthetic analogue CH8 in cutaneous leishmaniasis caused by *Leishmania amazonensis*. Here, we evaluate if these drugs are also effective against the African trypanosome, *Trypanosoma brucei brucei* 429 strain, which causes sleeping sickness in mice similar to the human illness. As a negative control we used CH3, a chalcone with no activity against *L amazonensis*. The *Trypanosoma brucei brucei* procyclic forms were plated at $5x10^3$ parasites per well and incubated with chalcones at concentrations of 0, 2, 20 and 200uM for 48h at 37^o C. The DMC and the CH8 exhibited similarly high antitrypanosomal activity, with IC50 equal to 2,1 ± 0,3 uM and 2,3 ± 0,2 uM, respectively. As found with leishmania, the CH3 was inactive on *T b brucei*, with IC50 higher than 200 uM. None of those chalcones were toxic for macrophages, as monitored by the release of the LDH cytoplasmic enzyme. Since they showed excellent selective inhibitory activity against both *L amazonensis* and *Trypanosoma brucei brucei*, it is conceivable that they act on the same parasite target, possibly shared by other kinetoplastid parasites such as *T cruzi*. This issue is presently been evaluated. Fiinancial support: CAPES

QT10 - The anti-leishmanial effect of Brazilian propolis ethanolic extracts

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Propolis is a resinous substance that honeybees collect from different plant exudates and it phenolic constituents possesses a variety of biological properties including antiinflammatory, antibacterial, antiviral and antiprotozoa activities. Propolis is widely used also in products like "health foods". The therapy of all forms of leishmaniasis require potentially toxic and painful multiple injections of pentavalent antimonials. On the basis of these facts, the present study was designed to investigate whether ethanolic extracts from Brazilian propolis collected in Alagoas (Marinho), Minas Gerais (BRP1) and Paraná (BRPG and BRG), were active against *Leishmania amazonensis* infected macrophage cultures[a]. The treatment of amastigotes infected macrophages with Marinho, BRP1, BRPG and BRG led to a decrease of infection and of the amastigotes proliferation. These infection reductions were about 100% for Marinho (12μg/ml) and BRP1 (100μg/ml), 80% for BRPG (25μg/ml) and 85% for BRG (25μg/ml). Comparation between the four propolis ethanolic extracts against *L. amazonensis* infected macrophages showed that Marinho propolis extract were more active than BRP1, BRPG and BRG. Noteworthy, these compounds did not exhibited cytotoxity against the murine host macrophages as tested by MTT assay. This is the first communication on Brazilian propolis ethanolic extracts as a new class of natural product with leishmanicidal activities, and encourages further investigation of the effect of Brazilian propolis in experimental *Leishmania*-infected mice. [a]Patent number (INPI: 018060007317). Supported by CNPq.

QT11 - Leishmanicidal and trypanocidal activity of plant products

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Leishmaniasis and Chagas disease affect mainly the poorest countries in the world. Despite the tremendous progress made in understanding the biochemistry and molecular biology of parasites, the therapy of protozoan diseases is limited to a reduced number of chemotherapeutic agents. Current treatment of leishmaniasis with pentavalent antimonials and Chagas disease with Nifurtimox or Benznidazole show chemotherapeutic failure and high toxicity and cost. Plants are a very rich source of new compounds that can be developed into new drugs for almost all diseases. Therefore, the search for novel, effective and safe therapeutic compounds has become a priority. In this work, we tested substances of several chemistry classes from different plants on *Leishmania amazonensis* (Josefa strain) and *Trypanosoma cruzi* (Colombian strain) growth. PMT1 and PMT2 extracts were isolated from *Piper marginatum*, L754 is an alkaloid isolated of *Cassia leptophylla* and XL1 is a diterpenoid isolated of *Xylopia landsfolia*. *L.amazonensis* promastigotes or *T. cruzi*epimastigotes were cultivated with 0-50or 0-50/ml of testing substances for 72h and controls were parasites cultivated with 0,06% DMSO. The parasite numbers were daily counted at Neubauer chamber. The most effective compounds against *L. amazonensis* were PMT2, XL1 and L754, which inhibited the number of promastigotes by 97%, 94%

and 63%, respectively. The results demonstrated a dose-related inhibition of parasites growth, with 50% activity at 25 /ml PMT2 and 65% at 50 XL1. *T. cruzi* epimastigotes were susceptible only to diterpene XL1, which inhibited 80% at 50and 60% at 25. Cytotoxicity against mammalian cells (resident mice macrophages) was evaluated for all substances (at 50 or 50 /ml). After 24 and 48h was not observed LDH release, suggesting that these substances do not have a citotoxicity effect on macrophages. Activity against intracellular amastigotes is currently assayed.

QT12 - Antileishmanial activity of plants used in traditional medicine in Brazil

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Leishmaniasis is parasitic protozoa disease and caused by different species of the *Leishmania* genus. This disease is regarded as a major public health problem, causing significant morbidity and mortality in underdeveloped countries. The chemoterapeutic of this disease has been based on the use of pentavalent antimonials. When this treatment is not effective, other medications used include pentamidine and amphotericin B. Considering the side effects and the resistance that pathogenic protozoa builds against these drugs, more attention should be given to the extracts and biologically active compounds which are isolated from plants species commonly used in herbal medicine. In this work, we tested methanolic extracts of folk medicinal plants to determine their *in vitro* antiparasitic effect against promastigotes of *L. amazonensis*, which has been associated with all clinical forms of leishmaniasis and *L. chagasi* which the causal agent of visceral disease. The extracts tested were: *Schinus terebinthifolius*, *Vernonia polyanthes*, *Cordia verbenacea*, *Rosmarinus officinalis*, *Cajanus cajan*, *Piper aduncum*, *Solanum americanum*, *Lantana camara*, *Ocimum gratissimum*, *Anacardium occidentale*, *Maytenus ilicifolia*, *Costus spiralis*, *Eugenia uniflora*, *Plantago lanceolata*, *Solanum concinnum*. 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. Cytotoxicity against mammalians cells was also evaluated for all compounds. Among the extracts assayed, *S. terebinthifolius*, *V. polyanthes*, *C. verbenacea*, *R. officinalis*, *C. cajan*, *P. aduncum*, *S. americanum* and *L. camara* inhibited the growth of *L. amazonensis* (IC_50 values of 55, 61, 120, 44, 62, 29, 40 and 14 μg/ml, respectively) while only the extract of *O. gratissimum* (flowers) was active against *L. chagasi* (IC_50 values of 71 μg/ml). None of the compounds were found to have significant toxicity towards mammalian cells at the maximal concentration used (250 μg/ml). Supported by FAPEMIG, BIC/UFJF.

QT13 - Antileishmanial activity of some 6-substituted purines

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Leishmaniasis, parasitoses caused by organisms of the LEISHMANIA genus, comprises three clinical forms: visceral, cutaneous and mucocutaneous. These infections are associated with significant rates of morbidity and mortality throughout the world. 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 explore the effect of several synthetic compounds against promastigotes of L. AMAZONENSIS and L. CHAGASI. The compounds tested were: 6-(3-chloropropylthio)purine; 6-(3-(N-cyclohexanamine)propylthio)purine ; 6-(3-(N-ethyl enediamine)propylthio)purine; 6-(3-(thioethylamine)propylthio)purine; 6-(alfa-ethyl acetatethio)purine; 6-(alfa-aceticacidthio)purine; 6-(4-(isoindoline-1,3-dione)butylthio) purine; 6-(pyridin-4-yl)methylthio)purine; 5-desoxy-5-iodo-1-orto-methyl-2,3-O-isopropylidene-beta-D-ribofuranose; 6-(5-Deoxy-1-orto-methyl -2,3-O-isopropylidene-beta-D-ribofuranose)purine; 6-(6-deoxy-1-O-methyl-beta-D-ribo furanose)purine; 6-(6'-Deoxy-1',2',3',4'-diisopropylidene-alfa-D-galactopyranose)purine; 6-(1'-Deoxy-2',3',4',5'-diisopropylidene-alfa-D-psicopyranose)purine. 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 (IC50) after three days incubation period. Cytotoxicity against mammalians cells was also evaluated for all compounds. Among the 13 tested compounds, four, 6-(3-chloropropylthio) purine ; 6-(3-(thioethylamine)propylthio)purine; 6-(alfa-aceticacidthio) purine and 6-(6-deoxy-1-O-methyl-beta-D-ribofuranose)purine showed an activity against promastigote forms of L. AMAZONENSIS (IC50 values of 50, 50, 39 and 29 microM, respectively) and anyone of the compounds showed an activity against L. CHAGASI. None of the compounds were found to have significant toxicity towards mammalian cells at the maximal concentration used (227 microM). Supported by FAPEMIG, BIC/UFJF.

QT14 - Antileishmanial activity of Ethanolic Extract and some compounds isolated from *Combretum leprosum* against *L. amazonensis*

promastigotes

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Recently, chemotherapy of leishmaniasis is still based on pentavalent antimonials diaminas and antifungal polyene these drugs are in general toxic, expensive, prone to generate resistance and require long-term treatment, which complicate the conclusion of the treatment. Therefore, there is a great and urgent need for the development of new, effective and safe drugs for possible leishmaniasis control. In this work, we tested the ethanolic extract 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 antileishmanicidal 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^{th} days at 23^{o} C. The pentamidina was the control. The development of the parasites was monitored due the daily counting in a microscope with a neubauer haemocytometer with Eritrosina B. Whereas the CLEFT-01 is very potent in inhibiting promastigote growth at 2μg/ml. The modified derivates compounds CLEFT-06 e CLEFT-07 showed less activity against promastigote forms (IC50 value = 5μg/ml in both compounds); and CLEFT-04 did not showed effect against them. The most effective concentration of the *C. leprosum* extract was 100μg/ml that reduced in 100% the number of promastigotes. Cytotoxicity against murine macrophages was evaluated for all compounds (5μg/ml) and the extract (50μg/ml), but none showed cytotoxicity. This is the first report of leishmanicidal activity of compounds isolated of *C. leprosum*. These preliminaries results indicate that extract of *C. leprosum* and its compounds could be used as a powerful tool for the development of new arsenals for the therapy of protozoan diseases. Financial Support: CAPES, CNPq

QT15 - In vivo testing of perillyl alcohol and perillyl aldehyde in the treatment of cutaneous leishmaniasis

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In order to identify new drugs to overcome current difficulties found in leishmaniasis chemotherapy, our group is testing the activity of terpenes, which are antimicrobial compounds produced in plants, against *Leishmania*. The activity of the monoterpene limonene against cancer and *Leishmania* has already been established. Promastigotes and amastigotes of *L. amazonensis* are killed by limonene with IC50s of 258 ± 4.9 and $147.3 \pm 4.6\mu$M, respectively. Limonene is quickly metabolized in the liver of mammals to perillyl alcohol (POH) and perillyl aldehyde (PCO). In vitro experiments in several systems have shown that POH exhibits stronger antitumoral activity than limonene. POH seems to exhert its antitumoral activity through inhibition of protein prenilation and apoptosis. We have previously shown that POH and PCO are active against *L. major* in vitro. In this work we show that POH and PCO are also active *L. amazonensis*. POH killed *L. amazonensis* promastigotes and lesion-derived amastigotes with IC_{50S} of 0.8 ± 0.08 and 5.2 ± 0.18 mM while calculated IC_{50S} of PCO were of 68 ± 4 and $152 \pm 40\mu$M against promastigotes and amastigotes, respectively.

In vivo toxicity studies showed that mice treated topically or intrarrectally with POH or PCO for 15 days did not present systemic or local side effects. Based on these tests, *L. amazonensis* infected BALB/c mice were treated with POH for 3 weeks. Treated animals showed a significant therapeutic response with a delay in the appearance of lesions. These mice will be followed up to verify if the response is long lasting.

In conclusion, limonene metabolites are active against *L. amazonensis* as well as against *L. major*. However, POH and PCO did not exhibit superior in vitro activity against *Leishmania* as compared to limonene. Their activity in vivo for the treatment of cutaneous leishmaniasis is under investigation.

QT16 - *In vitro* leishmacidal activity of an extract from the brown algae *Stypopodium zonale*.

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Infections due to Leishmania are a concern worldwide health problem, with high endemicity in developing countries. As there is no vaccine, the compounds in use for leishmaniasis treatment were expensive and toxic, there is still a strong need for efficient drugs. Here, we report the antileishmanial activity an dichlorometane extract obtained from the Brazilian brown algae Stypopodium zonale (SZE) collected at Marataises, ES. In a preliminary screening, we evaluated the possible leishmanicidal activity against promastigotes of Leishmania amazonensis. Our results demonstrate that SZE presents a dose- and time-dependent anti-L. amazonensis promastigotes activity. A 99% inhibition of promastigote growth was obtained with 10 ug/ml of SZE, this effect was perceived 24 hs after treatment. The reversibility of the effect on promastigotes was tested by incubating

parasites with SZE at different times, followed by culture in fresh medium. The SZE effect was not reverted as treatment with 10 ug/ml of SZE reduced parasite growth by 95% after 24 hs, achieving 100% after 72 hs of incubation in the fresh medium. The leishmanicidal activity was further evaluated in L. amazonensis-infected macrophages by adding SZE in the first day of culture. An 84% inhibition of amastigotes survival was seen using 10 ug/ml. It appears that our findings are the first report of S. zonale extracts exhibiting antileishmanial activity. Supported by: CNPq, CAPES.

QT17 - SCREENING OF NATURAL PRODUCTS ON *Leishmania amazonensis*

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Leishmania sp. are protozoan parasites, belonging to the Trypanosomatidae family , responsible for a group of diseases whose symptoms range from mild cutaneous lesions to fatal visceral involvement. Currently, treatment is based on a limited number of chemotherapeutic agents that are rapidly becoming ineffective and present high toxicity and cost. The search of new leishmanicidal compounds became extremely necessary. Natural products from the Brazilian flora compose valuable tools in the search for new antimicrobial agents. Here tested effects of the methanolic extracts from *Lippia alnifolia*, *Calliandra falcipita*, *Myrcia ferruginea*, *Mikania luetzelburgu*, *Norithanmus ganhophylus* and *Hyptis crassifolia* collected at Chapada Diamantina, Bahia state, against promastigote forms of *Leishmania amazonensis*. Samples were collected every 24 hours and counted on Neubauer chambers. At 24 hs, the *H. crassifolia* and *M. ferruginea* plant extracts at 100 μg/mL inhibited 65 % and 46 %, of the promastigote growth, respectively. At 48 hs treatments with extract of *H. crassifolia* we notice 89% inhibition of the growth of promastigotes. Ultrastructural analysis carried out for elucidate the cellular targets of action of these extracts on parasite survival is being. Further studies may provide new therapeutical compounds for leishmaniasis treatment. Supported by FIOCRUZ, CNPq, PROCAD/CAPES and FAPESB.

QT18 - EFFECT OF ITRACONAZOLE ON THE ULTRASTRUCTURE AND ENDODYOGENY OF *TOXOPLASMA GONDII*

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The antifungal agent itraconazole (ITZ) is an effective drug against systemic fungal infections inhibiting cytochrome P-450-mediated ergosterol synthesis in fungal membrane. Previous studies of our group demonstrated the high susceptibility of *Toxoplasma gondii* tachyzoites to azasterols and quinuclidines (Dantas-Leite *et al*, 2005 and Martins-Duarte *et al.*, 2006), known inhibitors of the ergosterol biosynthetic pathway. In this work we present data of the activity of this azole as potential agent for the treatment of toxoplasmosis. Monolayers of LLCMK2 epithelial cells infected with tachyzoites of RH strain were incubated with different concentrations of itraconazole for 24h and 48h. The IC_{50} values obtained were 114 nM and 53.6 nM respectively, demonstrating a selective effect of this drug against *T. gondii in vitro*. Transmission electron microscopy analysis of intracellular tachyzoites after treatment with itraconazole showed drastic mitochondrion swelling associated with an electron-lucent matrix and disrupted cristae. The drug also affected parasite endodyogeny producing tachyzoites containing many daughter cells still gathered. The drug is possibly affecting the budding process. This observation was confirmed by fluorescence microscopy after incubation of treated parasites with DAPI. Preliminary results have demonstrated that this drug might also be effective against chronic toxoplasmosis in murine models as the treatment with ITZ 10mg/kg reduced the numbers of cysts compared with the control group. Associative tests with antifolates are currently under study. This work was supported by CNPq, Pronex-Faperj.

QT19 - Antiparasites effects of Mercuric Chloride on intracellular Toxoplasma gondii

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Heavy metal has been widely used as fungicide and microbicide in pharmacology manipulation to produce the commercial drugs (1). Heavy metal as mercury, lead, cadmium and chrome were also used supporting of coadjutant of therapeutics drugs (2). For instance mercury chloride was used in combination of anticancer drugs. Meantime this purpose for antiparasites drugs was no studied. In present study we initially analyzed the citotoxicity effects of mercuric chloride on intracellular development of *Toxoplasma gondii*. Vero cells were cultivated in Linbro tissue plates and maintained at 37 °C and 5%CO2. Cultures were infected with tachyzoites at multiply 5:1 for 24 hours. HgCl2 Stock solution (0,1M) was prepared in distilled H2O, diluted in 199 medium for final concentrations of 8uM and 10uM and incubated in infected

cells for 24 hours. Cultures were fixed with Bowin solution, stained with Giemsa and examined at Zeiss Axioplan photomicroscope. During mercuric incubation infected cells decreased. At 8uM the percentage of infect cells reduced 20% while in 10 uM the reduction was 50%, resulting of the parasites elimination. At 8uM intracellular parasites decreased 40% while in 10uM this reduction represented 60%. Morphological analyses showed parasites in untreated cells with a crescent shape, forming a rosacea structure inside parasitophorous vacuole. However, in treated cultures intracellular parasites showed morphological alterations, where they were disrupted and disorganized inside the PV. These alterations were more drastic during incubation with 10uM and they can be related with parasites elimination. No morphological effects were observed on host cells. References: [1] Azevedo,A.F 2003. RiMa [2]Hessel.L. 2003. Bull Acad Nati Med 187(8):1501-10

QT20 - *Azadirachta indica* and *Melia azedarach* arrested and leaded elimination of intracellular *Toxoplasma gondii*

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Melia azedarach 'melia' and *Azadirachta indica* 'neem' (Meliaceae) extracts have several active biologically compounds with unknown molecular action. However these extracts have been used against insect, virus, bacterium, fungi, protozoan and nematodes [1,2,3]. Moreover, studies about extracts action anti-*Toxoplasma gondii* are inexistent. In present study we analyzed the effects of Neem and Melia on intracellular *T. gondii*.

Vero cells were cultivated in linbro tissue plates contained a sterile coverslip and maintained at 37°C, 5%CO2 overnight. Cultures were infected with tachyzoites of *T. gondii* (5:1) for 24hs. Extracts were obtained by leaves infusion in water at room temperature for 12h, reduced to powder and diluted in DMSO. This solution was sterilized by filtration in membrane of 0,22μm and added to 199 medium to final concentrations (500, 1000, 2000, 3000, 5000 ug). Infected cultures were incubated with extracts for 24h, fixed with Bouin solution, stained with Giemsa solution and examined at Zeiss AXIOPLAN photomicroscope using a 40x objective. Infected cells, parasites and parasitophorous vacuole (PV) number were determinate.

Following extracts incubation, the percentage of infected cells decreased. This reduction had been observed using 500ug of Melia extract; however the same effect only was observed with 2000ug of Neem extract. During incubation with both extracts in these concentrations, altered parasites were observed inside PV. Number of PV containing altered parasites increased during incubation with 3000μg of both extracts; however in higher concentration (5000μg), intravacuolar parasites were eliminated. These results demonstrated that Melia and Neen extract leaded to progressive elimination of intracelular *T. gondii*. Though, no citotoxic effects were observed at the host cells during incubation with both extracts.

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Support: CNPq; FAPERJ

QT21 - Characterization biochemical in the biosynthesis of thiamine in *P. falciparum*.

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Vitamin B1 (thiamine) is an essential cofactor for several key enzymes of carbohydrate metabolism. Humans and other mammals are unable to synthesize vitamin B1. Biosynthesis of isoprenoid in *Plasmodium falciparum* depends on the methylerythritol phosphate (MEP) pathway. The first enzyme of this pathway 1-deoxy-D-xylulose-5-phosphato sintase (DXS) is dependent of thiamine. The metabolite 1-deoxy D-xylulose-5-phosphate (DOXP) is not only intermediate of the MEP pathway but is also involved in the biosynthesis of thiamine and piridoxol that was characterized by us. Herein we described the biosynthesis of thiamine in intraerythrocytic stages of Plasmodium falciparum. Synchronous *P. falciparum* trophozoite stage cultures were labeled with [1-^{14}C] sodium acetate and L-[^{35}S]cysteine for 16 hs and schizont stages were recovered and extracted for thiamine analyses by RP-HPLC and TLC analyses. For the first time, it is demonstrated the biosynthesis of thiamine in protozoa parasite. Thiamine, thiamine phosphate and thiamine pyrophosphate labeled metabolically were observed by RP-HPLC and TLC. Results need to be confirmed by mass spectrometric analyses. Its absence in the human host makes pathway very attractive as potential new target for antimalarial drug development. Key words: Malaria, *Plasmodium falciparum*, Thiamine, HPLC, TLC Supported by CNPq

QT22 - SEARCHING FOR THE OXYGEN ROLE IN EXPERIMENTAL CEREBRAL MALARIA

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The severe forms of malarial infection, such as cerebral malaria (CM), are the major cause of obits, leading to more than two million deaths annually. Severe malaria is a multifactorial phenomenon involving the cytoadhesion or sequestration of infected erythrocytes (IE) to different host receptors in deep vascular beds. The full mechanisms involved in the pathogenesis of CM is still not well understood, however current thought indicates that both expression of proinflammatory cytokines, such as TNF and IFN-γ, and capillary occlusion by IE sequestration might be involved. It is believed that both hypotheses are associated to the CM clinical outcomes, such as confusions, ataxia, coma and death. However, whether sequestration leads to oxygen delivery impairment (hypoxia), and whether administration of 100% pressured oxygen could reduce CM symptoms have not been evaluated. In this study, we use a mouse model of CM with *Plasmodium berghei* ANKA to investigated the expression of the hypoxia-inducible fator-1α (HIF-1α), a transcriptional factor that promotes expression of genes encoding proteins involved in adaptative responses under low oxygen availability, and the effects of a hyperbaric oxygen therapy (HBO) on murine CM manifestation. Immunohistochemical analysis of brain sections from mice with CM revealed the expression of HIF-1α, mainly in the hippocampus. Moreover, mice daily exposed to HBO (100% O_2, 3 ATA, for 1H) displayed higher levels of survival and reduces blood-brain-barrier alterations in comparison to the non-exposed *P. berghei* ANKA-infected animals.

QT23 - VALIDATION OF A *PLASMODIUM FALCIPARUM* PARASITE TRANSFORMED WITH GREEN FLUORESCENT PROTEIN (GFP) FOR ANTIMALARIAL DRUG SCREENING

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Despite the campaign to 'Roll Back Malaria', developed by World Health Organization, in 1998, the rates of disease and death caused by *Plasmodium falciparum* in sub-Saharan Africa are growing. Parasite resistance to most available antimalarials drugs has been implicated as one of the main factors in this disturbing trend. Thus, the developments of new antimalarial drugs as well as new assays that facilitate and accelerate the screening process are prerequisites in this field of research. Recently, our research group showed that the rodent-parasite *P. berghei* transformed with GFP is a sensitive tool for screening in vivo blood schizontocial drugs (SANCHEZ et al., Int J Parasitol 34: 485, 2004). Aiming to set up an in vitro protocol to screening antimalarials, we used *P. falciparum* line transformed with GFP (PfGFP, MRA-317, MR4), in which fluorescence can be rapidly detected by flow cytometry. First, we demonstrated that the quantification of PfGFP by flow cytometry was remarkably specific, being directly correlated with parasitemia, as detected by microscopic determination. Further, to validate PfGFP model for in vitro drug screening, we compare our protocol with the conventional uptake of $(H^3) - hypoxanthine$, analyzing dose-response curves for some of the major commercial antimalarial drugs (chloroquine, quinine mefloquine and sodium artesunate). On the basis of a side-by-side comparison, this PfGFP-based method showed results similar to those obtained with the standard radioisotopic method, being no significant difference obtained between $IC_{50}s$ of each drug tested. Finally, we have been maintaining PfGFP parasite in continuous culture for more then one year without any modification on fluorescence profile, which validates widespread application of this transfected parasite for chemotherapy studies. Of interest, this PfGFP parasite is a quality controlled malaria-related reagents that is available without cost to the malaria researcher community, including free-shipping to endemic countries (MR4, http://www.malaria.atcc.org).

QT24 - EVALUATION OF THE IN VIVO AND IN VITRO ANTI-PLASMODIUM ACTIVITY OF THE VIOLACEIN EXTRACTED FROM Chromobacterium violaceum

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Globally, malaria represents the most important and pernicious parasitic disease of humans, affecting more than 500 million people, and leading to 2 million deaths per year, especially children and pregnant women. In Brazil, mainly in the Amazon basin, more than 400,000 cases of malarial infections are reported each year. The deliberated use of antimalarials, especially, chloroquine (CQ) in the past 50 years has provided tremendous selection pressure on malaria par-

asites to evolve mechanisms of resistance. This drug resistance, reported particularly by *P. falciparum*, is responsible for global resurgence of this disease in the last years. Drug resistance have evolved so rapidly among all antimalarials tested so far, that the only exception are the artemisinins. Based on those facts, the quest for the development of new drugs and an effective vaccine has achieved another dimension. Violacein, a pigment isolated from *Chromobacterium violaceum* in the Amazon River, presents diverse biological properties, including activity against tumoral cells, microorganisms, fungus and protozoa. Here we showed that violacein have in vivo and in vitro anti-plasmodium activity. The antimalarial activities of violacein were determined in vitro against *Plasmodium falciparum* 3D7 and in *Plasmodium chabaudi*-infected mice. In vivo analysis have shown that violacein treated mice (0-10 days post-infection) significantly inhibit parasitemia ($> 64\%$, $P < 0,001$) in comparison to the non-treated mice. Moreover, when tested in vitro against *P. falciparum* blood stage forms violacein (1 μM) displayed an IC-50 (50% inhibitory concentration) 300 times more potent than the quinine. The in vivo and in vitro antimalarial activity highlights the potential of violacein to be used as a malarial chemotherapy.

QT25 - Effects of steroids of *Solanum nudum* in normal and infected- *Plasmodium falciparum* erythrocytes

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Solanum nudum (SN) is a plant used by traditional medicine in Colombian Pacific Coast. Antiplasmodial activity of SN-isolated sterols against *Plasmodium falciparum*, *P. vivax* and *P. berghei* have been reported previously, however their mode of action is unknown. After invasion of the red blood cell (RBCs) by *Plasmodium*, the functionality of RBCs plasma membrane is critical for the parasite. Membrane function can be altered by sterols which may be incorporated into lipid bilayer. The aim of this study was to determine the effect of six different *S. nudum* sterols (SNs) in normal and *P. falciparum*-infected RBCs. Preincubation of normal RBCs with SNs in parasite culture decreased new ring formation in 59.4%, 51.3%, 50%, and 40.2%, for diosgenone, SN1, SN2 and SN4 respectively. Isosmotic hemolysis assays of normal RBCs did not show hemolytic activity of the SN compounds and no effect of SN-2 and diosgenone on the ultrastructure of normal RBCs was observed by transmission electron microscopy. Additionally, there were no differences in the protein profile of normal and *P. falciparum*-infected RBCs membrane microdomains containing flotilin and stomatin, when treated with SNs. Moreover, of the six compounds evaluated, SN2 and SN4 were found to inhibit sorbitol-induced lysis in *P. falciparum*-infected erythrocytes in a dose-dependent manner and at a significant extent (approx. 80%). The results suggest that the SNs are not toxic to RBCs and that the antimalarial activity of the SNs could be related to inhibition of the new permeability pathways (NPP) induced by *P. falciparum* in the RBCs. This work was supported by Colciencias grant 1115-05-13667 and the Universidad de Antioquia, Medellín, Colombia, and CNPq.

QT26 - N-myristoylation and palmitoylation in *Plasmodium falciparum*: characterizing the proteins and investigating inhibitors

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Resistance of *Plasmodium falciparum* to most of the available anti-malarial is now widespread, and the development of new drugs is regarded as a priority by the WHO. This task requires a thorough knowledge of the biochemistry of *P. falciparum*, and new chemotherapeutic targets are being investigated. The co and pos-translational linkage of fatty acids (myristyc acid and palmitic acid), to proteins plays an important role in the metabolism of the parasite, and is a potential target for new antimalarial drugs. In order to identify myristoylated and palmitoylated proteins in *P. falciparum*, cultures of parasites were metabolically labeled with [^{3}H]palmitic acid or [^{3}H]myristyc acid for 18 hs and each stage (ring, trophozoite and schizont) was purified by Percoll gradient centrifugation. SDS-PAGE and 2D gel analyses showed the presence of a number of palmitoylated and myristoylated proteins in all intraerythrocytic stages. These proteins have apparent molecular weights from 6 to 150 kDa, with most proteins in the 150-100 kDa, 70-40 kDa and 28-20 kDa range. In order to elucidate the mechanism of these linkages and investigate potential inhibitors of these modifications, three drugs are being tested in our laboratory: tunicamycin (a competitive inhibitor of protein acylation and an inhibitor of the N-glycosylation), cerulenin (an inhibitor of the fatty acid biosynthesis) and thiolactomycin (an inhibitor of the condensing reactions of type II fatty acid synthase). The found IC_{50}s were 21.8, 22 and 10μM, respectively, for each drug and in these concentrations inhibition of the protein synthesis was not observed. Treatment of the parasite with these drugs and metabolic labeling will evidence the inhibition of the linkage, and therefore, the inhibition of the transferases. Supported by FAPESP and CNPq.

QT27 - Artesunate, a potent antimalarial against *Plasmodium falciparum* chloroquine resistant, mobilizes calcium (Ca^{2+}) from intracellular compartments of the parasite.

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Artesunate (ART), a trioxane isolated from the plant *Artemisia annua*, exhibits activity on different phases of the asexual intra-erythrocytic cycle being indicated to treat patients with chloroquine-resistant *P. falciparum*(PfCR). ART also acts on gametocytes, blocking malaria transmission. The mode of action of ART involves: (i) inhibition of PfATPase6, a Ca^{2+}-ATPase pump on the endoplasmic reticulum (ER) membrane (Krishna et al., Nature, 2003); (ii) reaction with heme, a main product from hemoglobin digestion, forming a reactive compound that acts on fosfolipids (Vennerstrom et al., J. Med. Chem., 2005). In the present report we identified the target of ART in the PfCR trophozoites from a synchronous culture of W2 clone using a cell permeant fluorescent Ca^{2+} probe (Fluo-4AM) and dynamic images obtained through a confocal microscope. Different concentrations of ART ($\leq$4ng/ml) were added to the cells, killing the parasites at concentrations >3ng/ml. At 2ng/ml, ART promoted an increase of citoplasmic Ca^{2+}, regardless of EGTA, a Ca^{2+} quelant, showing that ART exhibits an intracellular action. In the presence of thapsigargin, an inhibitor of Ca^{2+}-ATPase pump from the ER, ART still increased the citoplasmatic Ca^{2+}, suggesting another intracellular target. To investigate the action of ART on the digestive vacuole (DV), trophozoites were loaded with a fluorescent H^+ probe (BCECF-AM) that accumulates on acidic compartments of eukaryotic cells. ART was able to mobilize protons from the DV, altering the pH gradient. In conclusion, ER and DV are intracellular targets for ART on PfCR, suggesting two modes of action of this antimalarial.
Financial support from CNPq, FIOCRUZ

QT28 - Antimalarial activity of mefloquine and artesunic acid against *Plasmodium falciparum* chloroquine-resistant increased by combination with ciprofloxacin.

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The widespread of *Plasmodium falciparum* (Pf) multi-drug resistant parasites led WHO (2006) to recommend combination drug therapy as first-line treatment. Formulations containing an artemisinin compound are standard policy in most countries that experience resistance to conventional monotherapies. The antimalarial activities of combinations between mefloquine (MEFLO) or artesunic acid (ART) with ciprofloxacin (CIPRO) were now tested. CIPRO is a synthetic fluoroquinolone with a broad spectrum of activity against pathogens, which displays synergism with other compounds, being useful to treat human tuberculosis (Díaz et al., Int. J. Antimicrob. Agents, 2003). For tests in vitro two (Pf) chloroquine-resistant isolates, both sensitive to mefloquine, were used: strain BHz26/86, (PfBHz), and clone W2 (PfW2). Inhibition of Pf growth by the drugs was measured in relation to controls without drugs by levels of [^{3}3H]-hypoxanthine uptake. The half-maximal inhibitory response (IC_{50}, in ng/ml), estimated by a curve-fitting for the antimalarials alone, was for PfW2 and PfBHz, respectively: 1.6 and 5 for ART; 13.5 and 18 for MEFLO; whereas in the dual combinations (1:1) CIPRO+ART they were 1.5 and 3.3. For CIPRO+MEFLO PfW2 exhibited 11.8. For the triple combination CIPRO+ART+MEFLO (1:1:1) the IC_{50} were 2.4 (PfW2) and 3.5 (PfBHz). The statistical analysis of IC_{50} values shows that the differences observed were significant for all CIPRO combinations in relation to each antimalarial alone, thus, revealing a synergistic action with the antimalarials. In conclusion, lower doses of MEFLO and/or ART may be used when combined with CIPRO, useful for treatment of patients with Pf multi-drug resistant.
Financial support: CNPq, PDTIS-Farmanguinhos-FIOCRUZ

QT29 - DETERMINATION OF GIARDICIDE ACTIVITY OF METRONIDAZOLE ANALOGOUS BY A COLORIMETRIC METHOD

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Giardiasis is a major diarrheal disease spread throughout the world *Giardia lamblia*, its causative agent, infects mainly children. The disease can cause malabsorption syndrome that in poor countries is responsible to children deficit in mental and physical development. In these places treatment constitute the major prophylaxis action because of the poor sanitary conditions. Giardiasis treatment is not efficient due to drug resistance presented by *G. lamblia* strains. Thus, assessment of new drugs against *G. lamblia* is a very important subject in the disease context. Another problem is related to the models used to measure drugs efficiency. In this work we investigate the giardicide potential of some metronidazole (MTZ) analogous produced by chemical modifications. It was evaluated 4 new chemicals, MTZ-mesilate (MTZMs), MTZ-iodine (MTZI), MTZ-bromide (MTZBr), MTZ-amide ($MTZN_3$). The concentration that inhibits 50% of cell growth -IC_{50} and the minimum inhibitory concentra-

tion -MIC of each chemical were determined for the strain Portland of *G. lamblia*. Trophozoites in logarithmic phase of growth had been distributed in cell culture plates for association with chemicals. The incubation was carried out for 24 hours in CO_2 at 37C. To determine chemicals efficacy we used a colorimetric method compared to a conventional measurement by counting cells in Neubauer chamber. The colorimetric method show to be better to measure the chemicals efficacy and could be used as a standard for drug activity quantification. All of the chemicals analysed presented high giardicide potential. MTZ presented IC_{50}= 1.96 μM and MIC = 34.10 μM, MTZMs IC_{50} = 0.69 μM and MIC = 10.32 μM, MTZBr IC_{50}= 0.28 μM and MIC = 4.23 μM, MTZN3 IC_{50}= 0.7 μM and MIC = 13.47 μM and MTZI IC_{50} = 0.4 μM and MIC = 6.69 μM. Financial support: CNPq

QT30 - Effects of tomatine and tomatidine on *Phytomonas serpens*

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Some plants produce substances to their own defense against pathogens and predators. In *lycopersicon* species such as tomato, the main antimicrobial compounds is the steroidal glycoalkaloid α-tomatine. Tomatine consist of a mixture of two glycoalkaloids: α-tomatine and dehydrotomatine. Both compounds are present in all parts of the plant, but the levels of the compounds drastically decrease in the tomato fruit during ripening. The loss of saccharide side chain of tomatine forms the aglycone tomatidine. In the present study we describe the effects of tomatine and tomatidine as inhibitors of grown of *Phytomonas serpens*, membrane permeability, cell division and morphological changes. Assays of release of piruvate kinase (citoplasmatic enzyme) shown that 10μM tomatine increased the permeability of the plasma membrane. Tomatidine was unable to release piruvate kinase, suggesting that the drug did not permeabilize the plasma membrane. Tomatidine added to culture medium arrested the cell grown without apparent death of cells. The number *Phytomonas serpens* of alive continued constant for at least 2 days after the drug. It was found that tomatidine cause a significative reduction of cells in process of division as well as morphological changes with decrease of cellular length, vacuolization, and shortening of flagellum. These changes in morphology were different to the produced by tomatidine on cells resuspended in PBS. In this medium clusters of long and very thin cell were observed. It is concluded that tomatine kills *Phytomonas serpens*, by selective plasma membrane permeabilization, probably by binding to steroids present in the membrane. However tomatidine decrease the ability of cells to divide without apparent effects on the permeability of plasma membrane. Curiously, the morphological changes on the cells were different if tomatidine was assayed on cells in PBS or in culture media suggesting that some additional factor of the medium participates in the mechanism of toxicity.

QT31 - Study of the action mechanism of the microplusin, an antimicrobial peptide isolated from the cattle tick *Boophilus microplus*.

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Antimicrobial peptides (AMPs) have an important role in innate immunity against infection in species ranging from invertebrates to vertebrates. Recently, many studies have been done to elucidate the relative contributions of cytoplasmic membrane and intracellular targets on the lethal mechanisms of AMPs (*Antim. Pept. Immunob.*, *online*, 2006). In the present work, we are investigating the action mechanism of microplusin, a peptide from *Boophilus microplus*, with 10,204 Da, six cysteine residues and rich in histidine residues at the C-terminal end (*Develop. Comp. Immunol.*, 28: 198, 2004). Recombinant microplusin is active against Gram-positive bacteria and filamentous fungi. It was demonstrated that microplusin reduces the number of colony forming units (CFU) of *Micrococcus luteus* by two orders of magnitude. However, no membrane permeabilization was observed (Program and Abstracts, XXXIV Annual Metting of SBBq, 2005). In contrast, microplusin showed to be lethal to *Plasmodium gallinaceum* through membrane permeabilization (Gomes, D. 2005 - Dissertação de Mestrado do Depto. de Parasitologia do ICB II/USP). In this work, we verified that *M. luteus* incubated with microplusin up to 24h recovered its growth when the peptide was removed from the medium. Besides, analysis performed by transmission electronic microscopy, revealed that a culture of *M. luteus* incubated with the peptide presented some bacteria without cellular contents. We also tested microplusin in *M. luteus* using a culture medium supplemented with trace elements. We observed that its minimum inhibitory concentration was increased, indicating a possible chelating property of this peptide and consequent lack of nutrients to the bacteria. At the moment, we are investigating if microplusin interferes with the biosynthesis of proteins. In addition, we are investigating the leishmanicidal activity of this peptide. A preliminary assay with MTT showed that microplusin reduces the viability of *Leishmania amazonensis* promastigotes. Supported by FAPESP and CNPq.

QT32 - Azasteroles and Bisphosphonates as Alternative Compounds in Chemotherapy of Giardia lamblia

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Giardia lamblia is a protozoan responsible for giardiasis, a disease distributed worldwide. This parasite colonizes small intestine of hosts. There are two forms of *Giardia*: trophozoites (proliferative), and cysts (resistance). The drug commonly used is metronidazole, which causes a lot of side effects, besides its using is not approved by FDA. Metronidazole is also carcinogenic if administrated in high and/or repetitive doses, needed during the treatment of resistant *Giardia* strains. Because the resistant cases are incrementing nowadays, new approaches in chemotherapy of giardiasis are required. Zaragozic acids, squalene synthase inhibitors, and bisphosphonate, have been tested in some protozoan parasites and promising results were achieved. Killing and ultrastructural effects were demonstrated during treatment with some of these drugs. Here we treat *Giardia* P1 strain with the compounds 22,26-AZAsterole and EPIminolanosterole, known as sterol biosynthesis inhibitors, however recent discoveries showed that AZA also alters phospholipid biosynthesis. Bisphosphonate RISedronate, a prenyl transferase inhibitor, was tested too. Dose-response effects were noticed during proliferation. The IC_50 values were 7,00 μM, 0,17 μM, and 12,11 μM for AZA, EPI and RIS, respectively. Ultrastructurally, enlargement of the peripheral vesicles was observed. The encystment process was also induced; however, the encystation vesicles presented atypical sizes, larger than the normal ones. Clefts, known as portions of the endoplasmic reticulum that will accumulate cyst wall components, giving rise to mature encystation vesicles, was seen in the course of the treatments. Cysts were found with their walls loosen and either juxtaposed. These changes in cell cultures may imply that *Giardia* is suffering the drugs effects. Encystation process can leave to unviable cysts; trophozoites with their peripheral vesicles probably will not survive, as we can verify during proliferation essays. Therefore, Additional studies are required to a better understanding of what is happening in cultures when they are submitted to these promising drugs.

QT33 - Trypanocidal activity of *Pterodon pubescens* fractions: identification of geranylgeraniol as the active component

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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. From the ethanol extract of *P. pubescens* seeds (OEP) three fractions were obtained by sequential extraction (hexane/dicholormethane/ethyl acetate), and the hexane fraction, PF1, was further separated by HPLC, yielding PF1.1, PF1.2 and PF1.3. OEP and the fractions were assayed against *T. cruzi*, PF1.2 showed the highest activity in assays with both epimastigotes and trypomastigotes. As PF1.2 consists only of geranylgeraniol (GG-OH) (Silva et al. J Pharm Pharmacol 56:135, 2004), further experiments were performed with this terpenic alcohol. GG-OH caused a dose-dependent inhibition of the percent of infection in macrophages (IC50/2 d = 4.2±0.9 microg/mL). This compound inhibited epimastigote proliferation with IC50 values of 174 and 22 microg/mL for, respectively, 1 and 4 days. Ultrastructural analysis of treated epimastigotes demonstrated the formation of concentric membranar structures inside the mitochondrion, of myelin figures in the cytosol and endoplasmatic reticulum profiles surrounding organelles and cytosolic portions. Such alterations suggest induction by GG-OH of an autophagic process. It was also observed mitochondrial swelling and an important increase in the number of lipid inclusions. Flow cytometry analysis confirmed mitochondrial damage by labeling treated epimastigotes with rhodamine 123, and the increase in the lipid content by the use of Nile red. These findings encourage us to further investigate the effect of GG-OH on *T. cruzi*.

QT34 - TRYPANOCIDAL AND LEISHMANICIDAL *IN VITRO* EFFECT OF SOME SYNTHETIC CHALCONE AND RELATED COMPOUNDS.

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Leishmaniasis and Chagas′disease are parasitic infectious still treated with expensive drugs that present severe side effects and are frequently ineffective, emphasizing the importance to search new compounds against these diseases. Chalcones are molecules belonging to the flavonoid family and possess a broad spectrum of biological activities. In this work we determined the *in vitro* activity of eight synthetic chalcones and related compounds against culture forms of *Trypasoma cruzi* Y strain and *Leishmania amazonensis* 7504 strain. Epimastigotes and promastigotes forms grown in LIT medium + 10% FBS and Schneider′s medium + 5% FBS, respectively, and had their concentration adjusted to $5x10^6$

parasites/ml. Bioassays were carried out at 27°C for 72h incubating 180μl of the parasites suspension/well in the presence of the 20μl of each compound (1 to 500μM/ml), solubilized in DMSO. As controls, parasites were incubated with 1% DMSO or 100μM of benznidazole (*T. cruzi*) or 10μM amphotericine B (*L. amazonensis*) and with medium only. Activity of the compounds was assessed by counting the number of surviving parasites in Neubauer chambers. The obtained data were compared by ANOVA and CC_{50} and IC_{50} values were calculated by linear regression analysis. Among 8 chalcones tested, 2 (C12, M4) had no antiparasitic effect. Four chalcones (C1, C17, C19 and C26) showed trypanocidal activity wich IC_{50} range between 69.9 to 164.8μ Five compounds revealed significative leishmanicidal activity (C1, C4, C17, C19, C26) wich IC_{50} values ranged at 10.4 to 54.9μM/ml. Intracellular amastigotes screening and cytotoxicity to mammalian cells must be carried out in order to confirm the potencial leshmanicidal and trypanocidal of these chalcones. Furthermore, studies are in progress to determine the possible sctructure-activity relathionships.
Supported by: ProBIC/ProPPEC/UNIVALI

QT35 - Characterization of a hypothetical protein overexpressed in *Trypanosoma cruzi* populations resistant to Benznidazole

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Murta *et al.* (2002) investigated the differential gene expression in *T. cruzi* populations with *in vitro*-induced (17LER) and *in vivo*-selected (BZR) resistance to benznidazole (BZ). Using the microarray methodology, the authors selected the TcHipo gene encoding a hypothetical protein. This gene was overexpressed in the *T. cruzi* population resistant to BZ. Many sequences from *T. cruzi* genome are apparently non-identified genes that correspond to hypothetical proteins. In this present study, we investigate differences in the levels of TcHipo mRNA in *T. cruzi* populations susceptible and drug-resistant to BZ. The northern blotting profile of total RNA from *T. cruzi* samples hybridized with TcHipo probe revealed one transcript of 980bp. Quantitative analysis revealed that the *T. cruzi* drug-resistant population 17LER and BZR expressed 4 and 2-fold more TcHipo mRNA than drug-susceptible 17WTS and BZS, respectively. In addition, we cloned the TcHipo encoding region (504pb) into pGEX expression vector. The results of expression showed that *Escherichia coli* BL21 expressed a GST-fusion recombinant TcHipo protein with a relative molecular weight of 65KDa for the rTcHipo (40KDa) fused with GST (25KDa). This recombinant protein was used as an antigen for producing rabbit anti-rTcHipo polyclonal antibodies. Western blotting analysis of *T. cruzi* protein extracts probed with anti-rTcHipo polyclonal antibody revealed a unique polypeptide of 40KDa for all strains analyzed. Further studies will focus the TcHipo protein level analysis in the *T. cruzi* strains susceptible and resistant to BZ and bioinformatics analysis will predict a probable function for the TcHipo protein. Supported by FAPEMIG, CNPq and CPqRR/FIOCRUZ.

QT36 - Immunomodulatory effects of the miltefosine and benznidazole during the treatment of Balb/c mice infected with *Trypanosoma cruzi* (Y-strain)

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Miltefosine, initially used as an anti-tumoral agent, is now being used as an oral drug (IMPAVIDO^R) for treatment of visceral leishmaniasis in humans. Besides, its toxic activity has also been demonstrated against *Trypanosoma cruzi*, the causative agent of Chagas' disease. In this work, we studied the immunomodulatory effects of orally (daily for 15 days) administrated miltefosine (25mg/Kg) and benznidazol (100mg/Kg) in Balb/c mice (groups of 10 animals) after IP infection with 10^3 *T. cruzi* Y-strain trypomastigotes, and compared the results with those from untreated infected animals. As observed previously, treatment of infected mice with miltefosine or benznidazol promoted 100% survival and clearance of parasites from the blood. After 15 days of infection, animals were sacrificed and histological and histomorphometrical studies showed a great decrease in cardiac inflammatory infiltration and absence of amastigote nests. Accordingly, measurements of the serum cardiac isotype creatine kinase activity and immunohistochemistry analysis of heart sections indicated that treatment with both drugs attenuated cardiac damage and further led to decrease of $CD4^+$ and $CD8^+$ T cells percentage in cardiac inflammatory foci. While flow cytometry showed non-treated mice cardiac T cells phenotype as $CD62L^{low/-}$/LFA-1^{high}/$CD2^{high}$, this phenotype changed after treatment, becoming $CD62L^{med}$/LFA-1^{low}/$CD2^-$ after miltefosine and $CD62L^{med}$/LFA-1^{low}/$CD2^{high}$ after benznidazol treatment, respectively. Taken together, the results suggest that, beyond the direct toxic effects against the parasite, these drugs also induce immunomodulatory effects that may help to reduce the damage caused/induced during acute infection by *T. cruzi*. Supported by: CAPES, CNPq, Fiocruz and FAPERJ. victors@biof.ufrj.br

QT37 - Nanostructured drugs with potential activity against *Trypanosoma cruzi*

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The development of new drugs for the specific treatment of *T. cruzi* infection remains the major challenge for Chagas disease control and health care. The aim of this study is to investigate a technological development of new pharmaceuticals products in nanostructured form for Chagas disease treatment. For this, Miconazole nanocapsules were prepared by interfacial polymer deposition following solvent displacement method using 10μM and 30μM of drug. The formulations were tested "*in vitro*"using the Y (*T. cruzi II*) and Colombian (*T. cruzi I*) strains, considered partially resistant and resistant to benznidazole, respectively. For evaluation of anti-*T. cruzi* activity, the Miconazole nanocapsules, 10μM and 30μM concentrations, were added in Y or Colombian acellular culture (LIT medium) with 0,5 x 10^7 epimastigotes/mL. The cell densities were measured daily using Newbauer chamber until 10^{th} day after incubation. The experiments performed with Y strain showed an increased trypanocidal activity for the Miconazol nanocapsules when compared with the free drug. After 168 hours of incubation with nanocapsules or free drug, the parasite growth inhibition was 63% and 94% (10μM) and 94% and 98% (30μM), respectively. However, no parasite growth inhibition was observed in experiments performed with Colombian *T. cruzi* strain in spite of the drug and formulation used. Ours results showed that the miconazol nanocapsules concentration necessary to induce around 90% of growth inhibition of Y strain was 3 times lower (10μM) in relation to free drug (30μM), indicating that miconazol action on epimastigotes forms was increased when it was used in nanocapsules preparations.

QT38 - ALPHA-LAPACHONE DERIVATIVE EFFECTS ON TRYPANOSOMA CRUZI AMASTIGOTE FORMS.

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Chagas' disease, caused by the Trypanosoma cruzi, is an endemic health issue in Latin America. In this context, an intensive research program has been focused on the study of alternative natural and synthetic drugs for Chagas' disease treatment. Jorqueira et al (2006) investigated trypanocidal effects on T. cruzi epimastigote form and cytotoxicity in VERO cell line of several oxyranes structurally related to a-lapachone. This study puts oxyrane 10 at an advantage as it shows low citotoxicity with about the same b-lapachone trypanocidal activity. In this present work we describe oxyrane 10 effect on T. cruzi trypomastigote and amastigote forms. Material and Methods 1- Parasite- Trypomastigote forms of the Y strain of T. cruzi and Colombian were obtained by in vitro infection of LLC-MK2 cell lineage as described in Pinho, RT et al. (2001). 2- Substances- Oxirane compound was prepared in dimethyl sulfoxide (DMSO). Final DMSO concentrations never exceeded 0.1%. 3-Drug action analyses - Oxyrane final concentrations were 25mM, 50mM and 100mM and group control was treated with DMSO 0.1%. Peripheral blood mononuclear cells from health donors were separated by Ficoll-Hypaque gradient centrifugation. The macrophage cultures were infected at ratio 1/10 with Trypanosoma cruzi Y or Colombian strains for 3 h at 37^{o}C and the remaining parasites were removed washing three times the macrophages with RPMI medium. Oxirane effect was assessed on the 2nd and 3rd days of infection by counting infected cells, non-infected cells, and parasite numbers within cells with by optical microscopy. Conclusion-. Analysis showed a macrophages infection rate decrease of about 34 % to both strains. Additionally it was also observed that intracellular parasite number decreased 47% to Y strain and 53%* to Colombian strain. Results reveal that oxyrane has an extensive trypanocidal activity on amastigote intracellular forms.

QT39 - TRYPANOCIDAL EFFECT OF OXYRANE DERIVATIVE OF α LAPACHONE AGAINST *Trypanosoma cruzi* TRYPOMASTIGOTE FORMS.

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Chagas disease, caused by Trypanosoma cruzi, is an endemic health issue in Latin America. It is a very serious public health problem in several countries, with about 18 million people known to be infected with the parasite. In this context, an intensive research program has been focused on the study of alternative natural and synthetic drugs. Jorqueira et al - 2006 investigated trypanocidal effects on T. cruzi epimastigote form and cytotoxic in VERO cell line of several oxyrans structurally related to a-lapachone. This study puts oxyrane 10 at an advantage as it shows low citotoxicity with about the same b-lapachone trypanocidal activity. In this work we describe oxyrane 10 effect on T. cruzi trypomastigote form. Materials and Methods 1-Parasites- Trypomastigote forms of the Y strain of T. cruzi - Silva and Nussenzweig,

1953 - and Colombian - Federici et al., 1964- were maintained in vitro by infection of LLC-MK2 cell lineage as described in Pinho et al. 2001. The cell culture supernatants were harvested, the trypomastigotes were washed with RPMI medium containing 10% human serum and counted in Neubauer chamber. 2- Activity against trypomastigote- Stock solutions (5μM) of oxyran 10 was prepared in dimethylsulfoxide (DMSO). The trypomastigote forms were incubated with the compounds at two different concentrations (25, 50 and 100 μM) , each one in triplicate, in order to determine the lethal concentration against trypomastigote form. Alive T. cruzi trypomastigote forms from 1st, 2nd and 3rd days cultures, in RPMI medium contain human serum, were counted in a Neubauer chamber using an optical microscopy. Non-treated trypomastigote forms control corresponded to of parasite living. Results and Conclusion-. Analysis showed alive trypomastigote number decrease 97% to Y strain and 84% to Colombian strain. Results reveal that oxyrane has an extensive trypanocidal activity on trypomastigote forms.

QT40 - ANTITRYPANOSOMAL ACTIVITY OF NOVEL AMPHIBIAN CUTANEOUS SECRETIONS

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Amphibian cutaneous secretions represent a rich source of novel peptides and organic compounds for the screening of potential antiparasitic drugs. Chagas Disease is a long-lived disease and a serious public health problem, affecting about 18 million people in America. The currently available nitroimidazole is unsatisfactory due to frequent toxic side effects and not effective in the chronic disease. Leishmaniasis is endemic in 88 countries, most of them from Central and South America. It afflicts 12 million people in tropical and subtropical areas. For six decades, long parenteral courses of the toxic pentavalent antimonials have been used for the treatment of Leishmaniasis. New chemotherapeutics are urgent. In this work, we have in vitro studied the activity of the novel amphibian secretion, *Corythomantis greeningi*, *Physalaemus fuscumaculatus*, *Macrogenioglotus allipioi*, *Dermatonotus muelleri*, *Siphonops annulatus* and *Bufo crucifer* against *Leishmania L. chagasi* and *Trypanosoma cruzi*. Two cutaneous secretions presented a high activity against *Trypanosoma cruzi* tripomastigotes: *Corythomantis greeningi* with an EC50 of 45.15 μg/mL (95% Confidence Interval - 25.07-81.30 μg/mL) and *Siphonops annulatus* 102.60 μg/mL (95% Confidence Interval 93.39 -112.70 μg/mL). No cutaneous secretions presented considerable activity against neither promastigotes nor intracellular amastigotes of *Leishmania (L.) chagasi* at the highest tested concentration of 500 μg/mL. Considering the 50% Effective Concentration of aqueous extracts of the amphibian secretions, we could suggest additional isolations in order to separate active substances from the mixture of compounds usually found in crude extracts. Further studies could be of interest for the study of new chemotherapeutics for Chagas Disease. These data was supported by FAPESP (Proj 2005/00974-9) and Instituto Butantan.

QT41 - IMPACT OF DUAL-CLONAL INFECTIONS ON TREATMENT EFFICACY IN BALB/c MICE INFECTED WITH *Trypanosoma cruzi* MAJOR GENOTYPES

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The impact of *T.cruzi* dual-clonal infections on benznidazol (BZ) treatment efficacy compared with the respective monoclonal infections was investigated. For this, eight clonal stocks, two of each major genotypes 19 and 20 (*T.cruzi* I), hybrid genotype 39 and genotype 32 (*T.cruzi* II) were combined into 24 different mixtures. BALB/c mice were inoculated by intraperitoneal route, with 5.000 blood trypomastigotes of each clone and treated with oral doses of 100mg Benznidazol/Kg/20 days. The cure control was performed by fresh blood examination, hemoculture, PCR, ELISA and detection of anti-live trypomastigotes antibody. The identification of each clone from not cured mice was performed by microsatellites assay. Cure in dual-clonal infection was detected in 28,4% of treated animals. Considering the cure rates for *T.cruzi* I (35,4%), *T.cruzi* II (60%) groups and their associations were not observed difference in relation to the expected BZ susceptibility for combinations *T.cruzi* I+I (0%), I+II (22,1%), except II+II (60,0%). For major genotypes, combinations 19x32 (26,7%) and 19x39 (25,6%) shiped their phenotypes to resistant profile and 39x32 to susceptible profile (60,8%). Genotype 20 was 100% resistant to BZ in monoclonal infections, but the cure rates of their combinations ranged from 0 to 24,5%. Nine out of 24 dual infections changed their profile of BZ susceptibility: 20+39 (n=2), 20+32 (n=1), 19+39 (n=3), 19+32 (n=2) and 39+32 (n=1). Although molecular characterization had identified few mixed infections in isolates from not cured mice, very interesting results were observed. In some mixtures (sensitive+resistant), the selected clone identified after BZ treatment was that previously identified as susceptible in monoclonal infections. These results suggest that mixed infections, so current in nature, may have important impact on chemotherapy efficacy. Futher studies to elucidate the mechanisms involved in this process are essential for advances in the knowledgment of Chagas disease chemotherapy. **Supported by FAPEMIG, CNPq, UFMG and UFOP.**

QT42 - Synthesis and Evaluation of Novel Mesoionic Compounds Against *Trypanosoma cruzi*

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Mesoionic systems have provided numerous compounds with wide-ranging biological activities. In a previous work, we carried out a comparative study involving chemical structure and biological activity using piperine, an amide isolated from *Piper nigrum*. In that study, the importance of both the unsaturated carbon chain and the amide function for the toxic effect on *T. cruzi* were demonstrated. Now, the methylenedioxy function was maintained and chemical modifications have been made in the amide group of piperine. Four synthetic compounds (M1, M2, M6, M7) have shown a potent activity on epimastigotes ($IC_{50} < 0.4$ μg/mL). We have them monitored the viability of murine macrophages by trypan blue exclusion test to select non-toxic compounds against mammalian cells. Three compounds (M2, M6, M7) showed a non-selective toxic effect. Then, further studies were carried out with M1 to evaluate its toxic effect against amastigotes and macrophage culture-derived trypomastigotes. *T. cruzi*-infected murine peritoneal macrophages were treated or not with increasing amounts of M1 (0.5 - 10 μg/mL). The anti-amastigote effect was investigated by counting the number of amastigotes/cell and the trypomastigotes survival verified by counting of trypomastigotes released in the supernatant. M1 showed a potent action against amastigotes ($IC_{50} = 0.62$ μg/mL) and inhibited the trypomastigotes release with 0.5 μg/mL from the sixth day post-infection. To investigate the toxic effect of M1 *in vivo*, Balb/c mice were infected with a lethal dose of blood trypomastigotes (10^5/mice) and treated or not orally with M1 for fifteen days with a dose of 50 mg/Kg. The parasitemia was evaluated from the fourth day and mice survival was monitored for thirty days. The results showed that M1 was able to protect infected mice and to reduce parasitemia, presenting similar values to those of infected group treated with benznidazole (100 mg/Kg). Supported by: CNPq, CAPES, FAPERJ, FUJB and TWAS.

QT43 - Evaluation of etiological treatment efficacy with Benznidazol during acute phase in the development of chronic experimental infection in dogs

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The efficacy of the etiological treatment of chagasic patients with drugs including Nifurtimox and Benznidazol in induce parasitological cure or prevent the development of chronic Chagas disease is frequently controversial. The aim of this study is to evaluate the benefit of the etiological treatment administered during acute phase in prevention of severe heart lesions evolution during the chronic phase of the disease. For this 12 dogs were infected with Colombian (*T.cruzi* I), Y or Berenice-78 (*T.cruzi* II) strains, resistant, partially resistant and susceptible to Benznidazol, respectively. Animals were treated after detection of patent parasitemia with 7mg/Benznidazol/kg administered in two daily doses for 45 consecutive days. Animals were euthanasied 180 days after treatment and heart fragments were collected for histopathological analysis in Hematoxylin-Eosin, Masson Trichromic and anti-*T.cruzi* immunohistochemistry preparations. Based on hemoculture, PCR and serological tests, 0%, 100% and 75% of the treated animals inoculated with Colombian, Y and Berenice-78, respectively, were considered cured. All treated and cured animals showed a significant reduction of tissue lesions in relation to the control untreated group. On the other hand, among treated and not-cured group, two patters of histopathological lesions were observed: (1) not-cured dog inoculated with Be-78 showed inflammation, degeneration and fibrosis less intense than untreated dogs and more intense that treated cured dogs; (2) in not-cured animals inoculated with Colombian *T.cruzi* strain, the treatment leads to more serious lesions than the untreated animals suggesting that for this strain, the treatment increased the lesions. These results indicate that the treatment was effective in reduce the tissue lesions among Y and Be-78 infected animals independent of the cure, but induces the progress of heart lesions in those infected with Colombian. Taken together these data indicates that the effectiveness of Benznidazol in reduction of tissue lesions may be related, at least partially, to *T.cruzi* population.

QT44 - Activity of aromatic diamidines against intracellular forms of Trypanosoma cruzi "in vitro"

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Chagas′ disease is an important parasitic illness caused by the Trypanosoma cruzi. It affects 17 million individuals in

endemic areas of Latin America and current chemotherapy is unsatisfactory based on nitroheterocyclic agents justifying the screening of new compounds. Aromatic diamidines represent an important class of DNA ligands and due to their high anti-parasitic activity against many pathogens, we aimed to evaluate the effect of furamidine analogues (DB711, DB889, DB786 and DB702) against intracellular parasites. After 24 h/37^{o}C of T. cruzi infection (parasite: cell ratio 10:1, employing Y strain), lineages of Vero cells were treated or not for 2-48h with crescent concentrations of the drugs (0.3-32uM), fixed, stained and the data evaluated by light microscopy (LM). IC50 values of the endocytic index (EI - percentage of infected cells versus mean number of parasite per infected cell) were calculated. To analyze the toxicity of the compounds against mammalian cells, uninfected cultures were incubated for 24h/ 37^{o}C in presence or not of higher doses (10-96uM) of the drugs, and then their morphology (by LM) and viability (by trypan blue exclusion assay) were monitored. Our data showed that three out of the four compounds presented high anti-parasitic activity: After 24 h of treatment, the IC50 related to the EI was 0.33, 0.33 and 1uM for DB889, DB702 and DB786, respectively. DB711 presented IC50 values superior of 32uM. The toxicity analysis showed that incubation of host cells with higher doses of DB889, DB711 and DB786 resulted in only 20% of unviable cells while incubation with DB702 decreased viability in about 60% of cells. The results shows the high anti-parasitic activity associated to low toxicity in mammalian cells of two promissory aromatic diamidines (DB889 and DB786), which are now being assayed against bloodstream trypomastigotes to better characterize the effects of these furamidine analogues against T. cruzi.

QT45 - Comparative analysis of the activity of aromatic diamidines and di-guanyl hydrazoneone against trypomastigotes forms of *T. cruzi* in vitro

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Chagas disease is an parasitic disease endemic in areas of Latin America, affecting 17 million people. Its etiological agent is the protozoan *Trypanosoma cruzi*. Two drugs have been used for the treatment of clinical Chagas disease: nifurtimox and benznidazole, but the results obtained with both drugs are unsatisfactory, claiming for identification of new anti-parasitic compounds. The aromatic diamidines represent an important class of DNA ligands however, despite its high activity against several pathogens, aromatic diamidines such as pentamidine present toxicity justifying the search for new analogs. Then, in the present study we tested upon bloodstream trypomastigotes of *T. cruzi* "in vitro", the action of two aromatic diamidines (DB 1195 a furamidine analog and DB1196, a pure diamidine) and compared to the data of another dication, (DB1080) that is a di-guanyl hydrazoneone. In these assays, trypomastigotes (5x106 parasites/mL, Y strain) were incubated for 2-24 hours at 4^{o}C in the presence of increasing doses (0.1-96μM) of the drugs diluted in whole mice blood. The drug activity was then evaluated by the quantification of viable parasite at light microscopy using a Neubauer chamber and the IC50 values (drug concentration able to kill 50% of the parasite population) were determinate. Our data showed that as soon as after 2 hours of incubation, the diamidines presented higher activity as compared to the di-guanyl hydrazoneone: DB 1196 showed an IC50 =9.46μM, while IC50 for DB1080 was higher than 96uM. After 24 hours of incubation, the corresponding IC 50 values were 1, 1.03 and 7.37μM for DB1196, DB1195 and DB1080, respectively, confirming the higher activity of the former compounds. Our data show promissory activity of aromatic diamidines against trypomastigotes of *T. cruzi* and another studies are under way in order to text the activity of those compounds against intracellular forms as well as against different parasite stocks.

QT46 - Acute and long-term infection in vertebrate host can modify *Trypanosoma cruzi* susceptibility to benznidazole

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The influence of *Trypanosoma cruzi* maintenance in different vertebrate hosts during the acute or chronic phase of the infection on its biological characteristics was evaluated. To do so, Swiss mice were infected with five (Be-62A and B, and Be-78C, D and E) *T. cruzi* isolates obtained from different dogs infected with Be-62 and Be-78 *T. cruzi* strains (2 to 10 years of infection), both 100% sensitive to benznidazole (Bz). After the determination of Bz susceptibility of each isolate in the first blood passage in mice (BPM), *T. cruzi* populations were maintained in successive BPMs. The objective was to investigate the occurrence of new changes in their level of Bz resistance, and in other biological parameters during the *T. cruzi* maintenance in mice (acute phase). The Be-62A, Be-62B, Be-78C, Be-78D, and Be-78E isolates showed respectively 50%, 60%, 90%, 70% and 90% of resistance to Bz after isolation from dogs during the chronic phase of the infection. On the other hand, new alterations in the drug susceptibility phenotype were observed during successive BPMs. Two tendencies were observed: (1) stabilization in Bz resistance level in Be-62A (50 to 40% in 60 BPMs), Be-62B (60 to 80% in 60 BPMs) and Be-78C (90 to 90% in 60 BPMs); and (2) reduction in Bz resistance level in Be-78 D (70 to 20% in 60 BPMs) and Be-78E (90 to 10% in 60 BPMs). The long-term *T. cruzi* infection also led to alterations in other biological parameters in mice. In general, the

isolates showed less virulence than the parental strains after long term chronic infection. These results support the hypothesis that the type of *T. cruzi* maintenance may change its phenotype of Bz susceptibility, as well as its virulence. Financial support:FAPEMIG / CNPq / UFOP.

QT47 - Variation in the resistance pattern to benznidazole in relation to the maintenance of *Trypanosoma cruzi in vivo* and *in vitro*

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The aim of this study was to induce *in vivo* resistance to benznidazole using *Trypanosoma cruzi* populations sensitive to the drug. To this aim, *Swiss* mice were infected with five (Be-62A and B, and Be-78C, D and E) *T. cruzi* isolates obtained from different chronic Chagasic dogs infected with Be-62 and Be-78 *T. cruzi* strains, both 100% sensitive to benznidazole. Four benznidazole-resistant *T. cruzi* populations were selected after 2 to 11 successive cycles of treatment with benznidazole. After stabilization of the resistant phenotype, *T. cruzi* populations were maintained for six to 12 months without drug pressure: (1) by successive blood passage in non-treated mice, and (2) in acellular culture in LIT medium. New changes in the level of benznidazole resistance were demonstrated after maintenance of the parasite without drug pressure. All benznidazole-resistant *T. cruzi* populations maintained by successive blood passages in mice remained, until now, 100% resistant to benznidazole. However, greater difficulty in detecting therapeutic failure was observed, indicating an increase in parasite subpopulations sensitive to benznidazole in these *T. cruzi* isolates. On the other hand, the 100% benznidazole resistant Be-78C and E revealed 50% and 20% of susceptibility after the maintenance in culture medium for one year and six months, respectively. These results corroborate the hypothesis that the manipulation of *T. cruzi* may influence its phenotype of susceptibility to benznidazole in *T. cruzi*. This work still demonstrates *in vivo* induction of resistance to benznidazole in *T. cruzi* strains 100% sensitive to the benznidazole (Be-62 and Be-78) for the first time, and establish an experimental model suitable for the study of drug resistance mechanisms in *T. cruzi*.

QT48 - TRIAZOLIC-DERIVATES EFFECTS ON TRIPANOSOMA CRUZI EPIMASTIGOTE FORMS AND CELLS VERO

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Currently benznidazole is the only available therapeutic agent for Chagas disease in Latin America. In this context, an intensive research program has been focused on searching for alternative drugs. The biological activities of the synthetics triazolic-derivates have been intensively studied. In the present work we describe the effect of the new synthetic substances, triazolic-derivates, on epimastigote form of *T. Cruzi*. **Materials and Methods** 1- *T.cruzi* Dm28c epimastigotes were raised in BHI-medium. 2-Trypanocidal Assay - Stock solutions of Triazoldaal and Triazoldagal were prepared in DMSO and their effect at 50μM was determined after quantification of active parasites on the 72 h and 144 h of the culturing, by counting in a Neubauer chamber using optical microscopy (Olympus Bx41). DMSO 0.1% was used as negative control. 4- Cytotoxic on the Vero cells was described as reported by Ferreira VF *et al.*, 2006 (Bioorg Med Chem. 14(16): 5459-66). **Results and Conclusion** - Triazoldaal and Triazoldagal killed 23,82% and 28,35% of the parasites (epimastigotes forms) respectively, in a period of 72 hours; and 72,12% and 64,56%, respectively, in a period of 144 hours. We investigated the toxic effects of these molecules [12.5, 25 and 50 μM] on the Vero cells. Interestingly, the two substances were poorly cytotoxic to this cell lineage and the cytotoxic effect was reduced to zero at the intermediate concentration of these substances. Further tests will be made in order to determine the concentrations that kill 100% of the parasites.

Vetores - Vectors

VE01 - MORPHOLOGICAL CHARACTERISTICS OF THE SENSORIAL ORGANS OF THE ANTENNAE OF *Lutzomyia ovallesi* AND *Lutzomyia migonei* (DIPTERA: PSYCHODIDAE) FEMALES, VECTORS OF CUTANEOUS LEISHMANIASIS IN VENEZUELA.

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Lutzomyia migonei and *Lutzomyia ovallesi* are sympatric species, found in South America, especially in Venezuela, and are incriminated vectors species of *Leishmania braziliensis* and *Leishmania mexicana*. The sensillae are sensorial organs with different shape and functions across the insects body. They are also found in the antennae and are responsible for recognizing stimuli and could be involved with feeding, aggregation, mating and are also capable of noticing of odors, humidity and temperature. The object of this study was to morphologically analyze the sensorial organs of the antennae of the adult female *L. ovallesi* and *L. migonei*, using Scanning Electron Microscope. Our result shows that both species have a par of thin antennae composed of sixteen segments. It was possible to identify six subtypes of sensillae classified as: *squame, smaller trichoid, blunt-tip trichoid, coeloconic, chaotic and pointed-tip trichoid.* The first segment, the scape, has a triangular format and the presence of the *smaller trichoid and the blunt-tip trichoid* sensillae was identified. The second segment, the pedicel, is of a sphere-like shape and the sensilla present were the smaller trichoid and pointed-tip trichoid. Posterior to the pedicel, it was possible to visualize fourteen extremely thin flagellomeres covered with microtrichias. The first flagellomeres in both species is different from the others due to its superior size that is extremely long. In *L. ovallesi*, the *pointed-tip trichoid* sensillae was found, in all the flagellomeres, but in *L. migonei* this type of sensillae was only found from the fifth flagellomere to the fourteenth, being absent in the first four flagellomeres. The other flagellomeres were extremely similar in both species. Our morphological observations on the sensorial organs of the antennae could facilitate future taxonomic and philogenetics studies. Supported by: CNPq, Fapemig, Pronex and Fiocruz.

VE02 - Identification of Oxide Nitric Reductase Proteins in salivary Glands of *Lutzomyia longipalpis* and in *Leishmania* spp

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Sand fly saliva has immunomodulatory activities, in addition to its antihemostatic properties, and there is evidence that *Leishmania* parasites utilize this activity to facilitate their establishment in the vertebrate host. Several approaches have been undertaken in order to develop new therapies or vaccines against canine visceral leishmaniasis (CVL). Among these approaches is the use of *L. braziliensis* and salivary antigens as an anti CVL vaccine. The aim of the present work was to characterize a salivary gland protein (47.3 kDa) of *Lu. longipalpis* that is recognized by antibodies from dogs with CVL and oxide nitric reductase proteins of *Leishmania* spp genome database. Identification of aminoacid sequence and analyses with bioinformatic tools predicted that the 47.3 kDa protein presented similarity with the ′Putative Yellow Related-protein′ that acts in the constriction of blood vessels and in the lubrification of the blood-feeding apparatus of the sandfly. Several domains were identified as a MRJP domain (Major Royal Jelly Protein), a substance secreted used to trigger development of a queen bee from a larva bee, a Homeodomain that are generally proteins with specific binding to the DNA and an nitric oxide reductase N-terminal domain, which suggests that this protein might be involved in the inhibition of NO production in macrophages. In regard to proteins of *L. braziliensis* and *L. major*, ten new proteins presenting nitric oxide reductase domains have been identified as a possible sort-specific globular cytoplasmic proteins. Considering that many antigenic components in the salivary glands of *Lu. longipalpis* and in the genoms of *L. braziliensis* and *L. major* have still not been characterized in vitro, the present results and further detailed studies on these proteins will help to reach a better understanding of the biology of *Leishmania*,its survival in macrophages, as well as the identification of new antigens as potential vaccine candidates.
Support:FAPEMIG/CNPq/CPQRR-Fiocruz/ UFOP/UFMG

VE03 - Investigating the action of some haematophagous insects ′ saliva upon the alternative pathway of the complement system

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Several biological activities have been described in the saliva of haematophagous insects. The saliva of *Lutzomyia longipalpis* and of the triatomíneos (*Triatoma brasiliensis, Rhodnius prolixus* and *Panstrongylus megistus*) was capable of inhibit both classical and alternative pathways of the complement system. The objective of this work was to investigate the inhibitory activity upon the alternative pathway in order to disclose which point, in the cascade, is affected by the inhibitor present in the saliva of those haematophagous species. An anti-C3b monoclonal antibody was utilized to investigate if deposition of the component C3b (that occurs in the first step of the alternative pathway cascade), could be inhibited by the insects ′ saliva. In this ELISA-like assay, the alternative pathway was triggered by agarose, a polysaccharide previously deposited on the micro plate wells. Aliquots of saliva from all the insects above mentioned were capable of diminish C3b deposition on the agarose surface. The interruption of the cascade in its initial events could be very important to protect efficiently the midgut epithelium against complement attack after a bloodmeal ingestion. Considering the amplification effect along the complement cascade, it is reasonable to expect that salivary inhibitors would act in the initial events, becoming more efficient.
Financial support:FAPEMIG / CNPq / CAPES

VE04 - Lytic activity of *Serratia marcescens* on *Leishmania braziliensis* and *Leishmania chagasi.*

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The effects of different populations *Serratia marcescens* on the *Leishmania braziliensis* and *Leishmania chagasi* were investigated. *In vitro* experiments, comparing incubations of 1x10^8 CFU/ml, 1x10^7 CFU/ml , 1x10^6 CFU/ml of suspension of *S. marcescens* SM365, a prodigiosin pigment producer, and *S. marcescens* DB11, a nonpigment variant, with 250 x 10^4 of promastigotes of both protozoa demonstrated that (i) *S. marcescens* DB11 was enable to lyse both *L. braziliensis* and *L. chagasi*, (ii) kinetic of lysis of promastigotes of *L. braziliensis* caused by *S. marcescens* SM365 was proportional to the period of incubation and the different concentrations of bacteria, and (iii) only the higher concentration (1x10^8 CFU/ml) of *S. marcescens* SM365 was able in lysis of *L. chagasi* promastigotes, in the other concentrations of bacteria the number of parasites maintained stable. In another set of experiments we examined the incubations for 120 min of 1x10^8 CFU/ml of *S. marcescens* SM365 with 250 x 10^4 of promastigotes of *L. braziliensis* and *L. chagasi* in presence of different carbohydrates. D-glucose and D-galactose were unable to interfere with lysis of both flagellates, while D-mannose protected both protozoa of the killing effect of *S. marcescens* SM365. These results showed that the lysis of *Leishmania* induced by *S. marcescens* SM365 variant is dependent of D-mannose and strongly suggest that bacterial fimbriae are important for the lysis effect of the bacteria on these parasites.

VE05 - 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 worldwide. In Brazil, there is a large distribution of the disease, with recent epidemic outbreaks. 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 the identification of the *L. longipalpis* gut transcriptome, under conditions of infection or not by *Leishmania chagasi*. The cDNA libraries, constructed in lambda zap, were generated from RNA extracted from *L. longipalpis* gut 6 and 72 hours after blood meal, and 72 hours after infection with *L. chagasi*. These libraries were excised and a total of 3711 clones were sequenced, with 2520 sequences showing high quality, corresponding to 832,5 Kb. In the library derived from infected gut 491 singlets and 179 clusters were found. In the library derived from gut after 72 hours of bloodfeeding, 473 singlets and 83 clusters were found. In the library derived from gut after 6 hours of bloodfeeding, 327 singlets and 68 clusters were found. The 2520 sequences were annotated and several genes related with peritrophic matrix (PM) physiology, stress and blood meal digestion were found, such as PM mucin, trypsin, trypsin inhibitor, chymotrypsin, ferritin, among others. In this project, 317 sequences (21%) did not have Blast hits against the used databases (GenBank, Pfam, Conserved Domain Database (CDD), Gene Ontology (GO) and Refseq). These sequences will be re-analyzed for the identification of putative new genes. We expect to analyze differences between the libraries to detect putative genes re-

lated to early/late blood meal digestion or innate immunity.

VE06 - EXPRESSION OF TRYPSINS IN *LUTZOMYIA LONGIPALPIS*

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Visceral Leishmaniasis is a major public health problem. In the New World it is caused by *Leishmania chagasi*, and in Brazil *Lutzomyia longipalpis* is the main vector. Insect midgut enzymatic activity during blood digestion is one of the main obstacles which *L. chagasi* must surpass to succeed in establishing infection. Trypsins are the main proteases secreted in the insect gut, and transcription may vary along the blood digestion process. We are interested in studying molecular aspects of blood feeding, to characterize specific molecules that might have a significant role in parasite-vector interaction, and might be used in developing potential targets for new strategies in the fight against the spreading of leishmaniasis. We have isolated and fully sequenced two trypsin cDNAs (Lltrip1 and Lltrip2) from an expression library of blood-fed *L. longipalpis* midgut and their sequences are similar to other insect trypsins. Specific primers were designed in order to be used in RT-PCR to investigate trypsin differential expression. Our experiments showed that Lltrip1 transcription has a peak at approximately 12 hours after blood ingestion, and disappears after 48 hours. Lltrip2 has a high transcription (four times higher than Lltrip1) in non-fed female and in male insects. Its transcription decreases after blood ingestion. High transcription is also observed in females after 96 hours, when digestive process is finalized and there is no more blood in the gut. Considering that Lltrip2 has a high transcription in non-fed insects, it is possible that this trypsin is translated, stored in vesicles and released immediately after blood ingestion. We are presently studying trypsin immuno-localization in the insect gut, using an anti-trypsin antibody produced by our group. We are also using this antibody to determine the appearance of the enzyme over time, through western blots. This work was supported by PAPES-Fiocruz and IOC-Fiocruz.

VE07 - Further analysis of the surface leishmanolysin-like molecules of *Herpetomonas samuelpessoai*: proposal of the secretion mechanism, degradation of several proteinaceous substrates and relevance during the *Aedes aegypti* colonization

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A metallopeptidase of 66kDa is the major glycosylphosphatidylinositol (GPI)-anchored surface peptidase produced by *Herpetomonas samuelpessoai*, which shares common biochemical and immunological properties with leishmanolysin of *Leishmania amazonensis*. Here, we studied the mechanism of secretion of this metallopeptidase to the extracellular environment. In this sense, living parasites were pre-incubated with 1,10-phenanthroline or p-CMPS for 1h. Then, the supernatant was analyzed by gelatin-SDS-PAGE. The zinc-metallopeptidase inhibitor 1,10-phenanthroline inhibited the peptidase secretion in a dose-dependent manner, while the phospholipase C (PLC) inhibitor (p-CMPS) did not alter the secretion pattern. Additionally, anti-cross reacting determinant (CRD) antibody failed in recognizing any secreted polypeptide from *H. samuelpessoai*, while anti-leishmanolysin antibody recognized a polypeptide of 63kDa. To detect whether the released leishmanolysin-like molecule possessed an entire GPI anchor, we treated the cell-free supernatant (rich in metallopeptidase) with PLC and then probed with anti-CRD, which presented negative results. Collectively, these results suggest that leishmanolysin-like molecule was released from the *H. samuelpessoai* surface by proteolysis instead of phospholipolysis, in a similar mechanism that is observed in *Leishmania*. This extracellular metallopeptidase of *H. samuelpessoai* was capable of hydrolyzing several proteins (albumin, hemoglobin, mucin, casein and insect-gut-proteins), showing a wide substrate utilization, which might helps in the parasite nutrition. Additionally, promastigotes of *H. samuelpessoai* were able to colonize *Aedes aegypti* guts. Then, we tested the effect of different compounds in the interaction of *H. samuelpessoai* cells and explanted guts of *A.aegypti*. When parasites were pre-treated with metallopeptidase inhibitors (1,10-phenanthroline, EDTA and EGTA), PLC or anti-leishmanolysin antibodies (H50 and H52) we observed a significant reduction in the interaction with gut cells. Similarly, the pre-treatment of gut cells with purified leishmanolysin-like protein drastically diminished the adhesion process. These results strongly suggest the participation of homologues of leishmanolysin molecules in the interaction of *H. samuelpessoai* with the invertebrate host. Financial support: FAPERJ, CNPQ, FUJB.

VE08 - A comparative study of vectorial competence of Lutzomyia longipalpis sandfly, vector of the Leishmania chagasi, considering wild-caugth and colonized sand flies

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Sandflies are insect vectors of pathogens, causative agents of arbovirosis, bartonelosis and leishmaniasis in man, constituting serious health problems in tropical countries. *Lutzomyia longipalpis* (Lutz and Neiva 1912) is the only proven vector of *Leishmania chagasi*, the etiological agent of a life-threatening

form of the disease, the visceral leishmaniasis. The objective of this study was to compare the vectorial competence in colonized and wild-caught sandflies. The *L. longipalpis* sandflies were reared in a closed colony established in 1999 and maintained in the Laboratory of Medical Entomology, (Fiocruz-MG). This colony was started from flies collected in Lapinha cave situated 35 km from Belo Horizonte and then maintained according to conditions described by Killick-Kendrick et al. (1973, 1977). The wild-caught sandflies were collected at the same place and used in the same day. Two groups of adult female sandflies were allowed to feed in a feeder device with mouse blood mixed with *Leishmania chagasi* or *Leishmania major* (amastigotes or promastigotes). The fully engorged sandflies were separated and maintained on 50% sucrose *ad libidum* at 25 degrees Celsius and 95% of humidity. Seven days after the infection, the sandflies were dissected in under a stereoscope, observed in the optical microscope for the presence of parasites. The colonized sandflies infected with *L. chagasi*presented infection rates of 86% (when the infection was initiated with amastigotes) and 81% (when the infection was initiated with promastigotes). Differently, the wild-caught sandflies infected with *L. chagasi* presented infection rates of 20% and 58%, respectively. Similar results were obtained with *L. major*. These results showed the vectorial competence of *L. longipalpis* for *Leishmania* parasites changed after the colonization procedures. Now, our studies are processing in order to better understanding this phenomenon. Financial supported by Fapemig, Fiocuz and Pronex.

VE09 - Antennal sense organs of the leishmaniasis vectors *Lutzomyia intermedia*, *Lu. whitmani*, *Phlebotomus papatasi* and *Ph. duboscqi*: a comparative study based on scanning electron microscopy.

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Lutzomyia intermedia and *Lu. whitmani* are important cutaneous leishmaniasis vectors in the Americas, and *Phlebotomus papatasi* and *Ph. duboscqi* in Africa and Asia. As both pairs of species are morphologically very similar and sympatric in certain areas, in this study, we compared the types, number and size of female antennae sensory organs, in order to detect within- and between-pair differences that would help in their identification. Six sensilla types were observed: chaetic, squame, campaniform, basiconic, coeloconic (grooved and ′praying-hands′), and small and large trichoid (both fine- and blunt-tipped). The observation that basiconic sensilla are only found on *Phlebotomus*, and grooved coeloconic II and small fine-tipped trichoid sensilla are exclusive of *Lutzomyia*, makes them good characters for genus-level distinction. Likewise, sensillum number and antennal segment dimension separate the two genera. With regard to the distinction at the species level, within *Lutzomyia*, the grooved coeloconic II sensillum is only present on flagellomere I of *Lu. intermedia*, whereas the ′praying-hands′ coeloconic sensillum only occurs on flagellomeres II and III of *Lu. whitmani*. Within *Phlebotomus*, *Ph. duboscqi* lacks the 'praying-hands' coeloconic sensillum on flagellomere I; and *Ph. papatasi* lacks the small blunt-tipped trichoid sensillum on flagellomere XI, as well as the large fine-tipped trichoid sensilla on flagellomere X. The dimensions of the chaetic sensilla, particularly of those on flagellomeres XII and XIII, are even able to discriminate all four species at once. The present work revealed important differences in size, number, and types of sensilla among the four sandfly species examined, which can be used as additional taxonomic characters to help on their identification. Financial Support: Fapemig, Capes, Fiocruz, CNPq and Pronex.

VE10 - Studies, by RAPD-PCR, of *Lutzomyia (Nyssomyia) whitmani s.l.* (Antunes& Coutinho, 1939) (Diptera: Psychodidae: Phlebotominae) populations, vector of American Cutaneous Leishmaniasis in Brazil

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The sand fly *Lutzomyia (Nyssomyia) whitmani s.l* is incriminated as a vector of American Cutaneous Leishmaniasis (ACL), associated with *Leishmania (Viannia) braziliensis* and *L. (V.) shawi* transmission. However, beyond its ability to transmit two parasites, differences in the behavior between distinct geographicaly populations have been suggested that this sand fly would be a complex of sibling species. The exact identification of these populations is important for epidemiological studies of the ACL, allowing the better knowledge of the transmission cycles, considering that this sand fly species occurs in the great majority of the Brazilian States. In the present study the genetic variability of two populations of *L (N.) whitmani s.l* was evaluated: from Buriticupu, State of the Maranhão, transmission area of *L. (V.) shawi* and *L. (V.) braziliensis*, and from Ilhéus, State of the Bahia, type-locality and endemic area for *L. (V.) braziliensis*. The RAPD-PCR (Random Amplified Polymorphic DNA - Polymerase Chain Reaction) technique was used. The genomic DNA of sand flies from each locality was extracted and later amplified. The used thermal profile was: 1^o cycle of 95°C, 5 min; 40 cycles of 95^oC, 1 min; 36oC, 1 min; 72oC, 2 min. Amongst six primers tested, *P4* (5′-AAGAGCCCGT-3′) was used. Considering the analyses of the products amplified in agarose gel 2%, it was observed a band of 380 bp, approximately, in both populations. Otherwise, another band of 200 bp was observed in individuals from Buriticupu, only, having suggested an inter-populational differentiation. The observed polimorphism shows a highest number of regions amplified in individuals from Ilhéus, compared with ones from Buriticupu, another evidence suggesting that these populations are

not identical.
Supported by ENERPEIXE

VE11 - Identification of molecules potentially involved in *Lutzomyia longipalpis-Leishmania chagasi* interaction.

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Visceral leishmaniasis is a high mortality disease when left untreated. Approximately 500,000 new cases occur every year, mostly in poor and developing countries. In Brazil visceral leishmaniasis is caused by *Leishmania chagasi*, which is transmitted mostly by *Lutzomyia longipalpis*. After 72 hours of bloodfeeding on an infected host, procyclic parasites are seen adhered to the insect gut via flagella, and this interaction is vital for parasite transmission. Little is known about molecules involved in this interaction. In *Phlebotomus papatasi* there is a gut galectin that recognizes the lipophosphoglycan present on the surface of *Leishmania major* (Kamhawi et al, 2004). Ismach et al. (1989) developed an antibody against *Leishmania* flagellum, that Warburg et al. (1989) demonstrated to be capable of inhibiting the adhesion of *L. major* flagella to the gut of *P. papatasi*. This antibody recognized a protein called Flag, that was later seen to belong the a small myristoylated proteins (SMPs) family (Tull et al. 2004). In our laboratory we identified a *Leishmania* surface protein belonging to this family, that we called Sup. We are presently investigating the potential role of this protein in adhesion of *L. chagasi* to *L. longipalpis* gut. We are also interested in identifying other proteins that might have a role in binding. Proteins from *L. longipalpis* gut, dissected after 72 hours of bloodfeeding, were separated by SDS-PAGE and transferred to a nytrocellulose membrane. This membrane was incubated with either recombinant Sup or *L. chagasi* previously labeled with biotin. Interaction was visualized by avidin reaction. Preliminary results indicate the presence of two candidates, with 27KDa and 18KDa, that interact with the parasite, and a 14KDa that seems interact with Sup. We are presently also testing Flag. Proteins with a role in insect-parasite interaction are candidates for transmission blocking vaccine development.

VE12 - Interaction of *Leishmania chagasi* with a cell line derived from *Lutzomyia longipalpis*: effect of platelet-activating factor (PAF) and cell signaling modulators

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Lutzomyia longipalpis is the most important vector of American visceral leishmaniasis due to *Leishmania chagasi* in the New World. Platelet-activating factor (PAF) is a phospholipid with potent and diverse physiological and pathophysiological actions, including on the interaction between *Leishmania amazonensis* and mouse peritoneal macrophages in vitro. Here we describe the effects of PAF, as well as several cell signaling modulators, on the interaction of *L. chagasi* with a *Lu. longipalpis* cell line in vitro. *Leishmania (L.) chagasi* promastigotes were grown in a complex medium supplemented with 10% heat inactivated fetal calf serum (HI-FCS). Insect cell lines LL-5 from *Lutzomyia longipalpis* and C6/36 from *Aedes albopictus* were grown in MM insect medium supplemented with 10% HI-FCS. The insect cells were seeded on glass coverslips inside 24-well plates and grown overnight. *L. chagasi* promastigotes were added to the adhered insect cells in MM-FCS at a 10:1 ratio. The parasites and/or the insect cell lines were incubated for 30 min in the absence or in the presence of PAF and the inhibitors of G protein (pertussis toxin), PKC (BIS) and CK2 (TBB), before the interaction assays. After 2-h interaction, the insect cells were extensively washed with PBS, fixed with methanol and stained with Giemsa. Overall, there was a synergic effect of protein kinase inhibitors in combination with PAF. When the parasites were pre-treated with PAF there was an increase in the association indices and a decrease when LL-5 insect cells were pre-treated, as compared with control systems. Pertussis toxin did not induce any effect when LL5 cells were used, but it promoted an enhancement in the association indices in C6/36 mosquito cell line system. Supported by: CNPq, FAPERJ, CAPES.

VE13 - Reduction of reprodutive fitness and changes on yolk proteins expression on *Plasmodium gallinaceum* infected *Aedes aegypti*.

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Aedes aegypti mosquitoes, in addition to its importance as a pathogen vector, represents an outstanding model system for arthropod vector research due to the exceptional knowledge base amassed on its physiology, biochemistry and development. Parasites by definition cause harm; if the consequence of this harm increases its transmission, the parasite virulence will probably be selected. Points of contact between vector and parasites begin with the ingestion of the infective blood meal. Once infected, the insect immune system may be manipulated, circulating metabolite titers changed, vector reproductive success and longevity altered, and the process

of further blood feeding affected. In this study the expression of 3 genes, vitellogenin (VG), vitellogenic cathepsin B (VCB) and lipophorin (LP) were investigated during the first gonotrophic cycle in *Plasmodium gallinaceum* infected *Aedes aegypti*. The results obtained by Reverse Transcription-PCR and Real-Time PCR showed on VG and VCB transcription decrease about 50% and 30%, respectively. On the other hand, LP did not change the amount during vitellogenesis process, we are showing that the amount of VG, VCB and LP in the ovaries was reduced on infected females. Analysis of hemolymph ecdysteroid levels showed a significant reduction thirty hours after infected blood meal. We observed that on the infected females the egg production had a 26% reduction. We also show that 7-8 days after infected blood meal LP transcripts increased 60%. The hemolymph LP level at this period increases and it is coincident with late sporogonic stage, suggesting that the parasites need lipids to complete their development.

VE14 - Angiotensin II and synthetic related peptides are active against *Plasmodium gallinaceum* sporozoites

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Transmission of malaria parasites by mosquito vectors is dependent on the successful development of *Plasmodium* infective forms, particularly the sporozoites, which are the forms that enter the vertebrate host. Drugs able to kill these forms might be useful as malaria preventive and chemotherapic agents. If these drugs are peptides they may be also utilized to modify mosquitoes genetically in order to produce insects resistant to *Plasmodium* transmission. In our search we found that Ang.II is able to kill *Plasmodium gallinaceum* sporozoites (88%) without damaging vertebrate cells. After modification of the molecular structure of Ang.II, we obtained 6 related peptides named Vaniceres (VC 1 to 6). The effect on ileum contraction and ability to kill sporozoites were investigated for VC 1-6 and the results show that VC5 had no agonistic effects and is able to kill 76% of sporozoites in the condition utilized in our experiments. Since Ang.II and VC5 kill the *P. gallinaceum* sporozoites in the same way, by plasma membrane disruption and VC5 does not have this effect on vertebrate cells, this is a very promising molecule to be tested for malaria prevention and also chemotherapy. Improvements might be done to the Ang.II related peptides structure in order to optimize theirs actions. A great research effort must be done about these active Ang.II related peptides, since they are promising molecules that may contribute to control malaria, the most prominent disease caused by protozoan.

VE15 - Differential gene expression by *Plasmodium* and Dengue infected *Aedes aegypti*

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The development of transgenic mosquitoes expresing anthipathogen effector molecules and their ability to block or reduce disease transmission under laboratory conditions has fostered enthusiasm for releasing these insects as a tool to increase the efficiency of integrated insect vector control program. Selection criteria for genes for which promoters could drive sustainable expression of transgenesis include those that have a high level of constitutive expression. However, such expression patterns and product accumulation may have a negative physiological effect on larval and pupal stages when effector molecule expression is not needed. Furthermore, infections by different pathogens have been shown to reduce reproductive fitness of infected mosquitoes compared with uninfected ones. Using *Plasmodium gallinaceum* or Dengue (DEN-2) infected *Aedes aegypti* we have analysed differences in mRNA profiles that reflect changes in transcriptional activity of 57 genes during infection using a macroarray approach. By differential screnning we show that 3 transcripts are upregulated and 4 transcripts are downregulated on *P. gallinaceum* infected *Aedes aegypti*. On the other hand, dengue infected mosquitoes show to have less impact on transcription of those genes. Our results show that 2 transcripts are upregulated and 1 trancript is dowregulated. We are using quantitative analysis of transcripts by real-time polymerase chain reaction (qRT-PCR) for detect mRNA amount during a *P. gallinaceum* or dengue virus infections. We belive that quantitative real- time qRT-PCR is a convenient and reliable method that provides new insights into pathogens-vector interections. Supported by FAPESP and CNPq

VE16 - ENGINEERING PATHOGEN RESISTANCE IN TRANSGENIC MOSQUITOES

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We are working to develop transgenic mosquitoes that are resistant to malaria parasites and that can be used in the control of transmission of the disease. The developmental cycle of malaria parasites has three invasive stages, merozoites, which infect the vertebrate erythrocytes, ookinetes, which penetrate cells in the mosquito midgut, and sporozoites, which are distinguished by their ability to invade two types of cells, those in the mosquito salivary glands, and the primary targets in the vertebrate host. In an attempt to target sporozoites, we are studying molecules that interacted with the parasites by different mechanisms. We are using molecules

that are comprised of competitor peptides that bind to salivary glands receptors blocking sporozoite invasion. It is our intention to produce a gene that encodes Plasmodium ssp TRAP-domain A which have been assigned as playing major roles in the sporozoites invasion process (Crisanti, A.,et al., 1999). We cloned the three putative domains based on P.gallinaceum, P.falciparum and P.vivax TRAP sequences on transient expression system Sindbis vírus. The recombinant viral particles were recovered and the blocking assays will be discussed.

VE17 - ANATOMICAL MODIFICATIONS OF THE MUSCLE NETWORK IN *AEDES AEGYPTI* MIDGUT CAUSED BY *PLASMODIUM GALLINACEUM* OOCYSTIS DEVELOPMENT

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The infection of *Ae. aegypti* with *P. gallinaceum* has been considered a good model for developing studies related with the interaction processes between malarial parasites and vectors. The importance of this model has been increased due to the molecular studies that suggested genetic proximity of *P. gallinaceum* with *P. falciparum*, causative agent of human malaria. This study aims to verify possible morphological changes in the mosquito midgut after the infection with *P. gallinaceum* considering that the organ wall is the place where the oocysts development occurs. Midguts were dissected between 0-17 d after feeding with normal or infected blood and processed by two techniques: Phalloidin/FITC for actin labeling to unveil muscle fibers under laser confocal microscopy (LCM) and scanning electron microscopy (SEM). Samples were fixed in 4% formaldehyde for the actin labeling or in 2.5% glutaraldehyde for SEM. For the LCM, the midguts were incubated with Phalloidin/FITC for 2 h and with a nuclear marker, to-pro 3 overnight followed by mounting in a glass slide with Mowiol (anti-fading). For the SEM, samples were routinely processed. The Phalloidin labeled the sand fly muscle fibers demonstrating the presence of actin filaments. The ooscysts were well stained by to-pro 3 contrasting with the green muscle fibers. Our results demonstrated anatomical modification in the organization of the muscle network related with the arrangements of the muscle fibers. The entire muscle network was disturbed by the oocyst development. The SEM confirmed and showed details of these structural modifications. In conclusion, this study showed that the muscle fibers present as well-built muscle network in the *Ae. aegypti* midgut is suffering drastic morphological changes due to the infection with *P. gallinaceum* . Financial support: Fapemig, Pronex, CNPq, Fiocruz and CAPES

VE18 - *In silico* comparative analysis of *Anopheles gambiae* genes expression profile: A screening tool to identify *Aedes aegypti* later vitellogenic genes.

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Genetic modification of the vectorial capacity of mosquito vectors of human diseases requires promoters able to driving the effectors molecules expression with sex, tissue and stage specificity. Postvitellogenic genes promoters are also of potential interest. It can be used in the future for engineering transgenic refractory mosquitoes, with a minimal fitness load in vitellogenesis. In order to find such promoters, we are targeting the identification of differentially expressed genes, activated at the termination of *Aedes aegypti* vitellogenesis. An initial *in silico* analysis in *Anopheles gambiae* Gene Expression Database (http://www.angaged.bio.uci.edu/) permitted identify 22 transcripts that showed female and stage specific expression at 48h after a blood meal (PBM). The *Anopheles gambiae* transcripts sequences were analysed on the BLAST algorithm against the *Aedes aegypti* Gene Index (http://www.tigr.org/tdb/tgi/aegi/). These analysis provided eight *Aedes aegypti* sequences, founded four transcripts with predicted functions (NABZ660TF, TC36931, TC44745 NACN796) and four unknown function ESTs (TC38081, TC49042, TC47124, TC48021). We designed primers to the 8 sequences of *Aedes aegypti* and analysed stage and tissue specificity by RT-PCR. Five transcripts (NABZ660TF, TC36931, TC44745, TC47124, TC48021) showed transcription in all stages with a high expression 48h PBM. TC49042 transcript showed expression in pupae female and high expression 48h PBM, and two transcripts (TC38081 and NACN796) presented female specific expression activated 48h and 72h PBM. Moreover, the initial comparative RT-PCR tissues analysis indicated expression of TC38081 and NACN796 transcripts in fat body and a strong expression in the ovarian tissues of the *Ae. aegypti* female mosquitoes. The absolute quantitation of transcripts have been done using Real-Time RT-PCR to provide a better determination of transcriptional profile of these genes. The *in silico* comparative analysis of genes profile expression provided an interesting strategy to screening genes to isolating regulatory regions for use in transgenic mosquitoes. Supported by CAPES, CNPq and FAPESP

VE19 - Evaluation of basic aspects of development and reproduction of Neotropical Anopheles, malaria vectors, in laboratory

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Malaria is one of the parasitic diseases that more cause social and economical problems worldwide. It is endemic in Brazil, with 500,000 new annual cases. Malaria is transmitted by *Anopheles* mosquitoes and in Brazil *An. darlingi* is the main

malaria vector, mainly in the Amazon Region. *An. aquasalis* is a primary malaria vector in the Brazilian coast, due to its preference to brackish water. Other *Anopheles* species, such as those belonging to the Albitarsis Complex, composed of at least four cryptic species, are involved in malaria transmission. Although vector control is one of the main tools in the fight against malaria, it is not presently efficient, because of lack of basic knowledge concerning neotropical *Anopheles* biology. This is partly a consequence of the difficulty in the establishment and maintenance of these species in captivity, Since 1993, our Laboratory maintains neotropicals *Anopheles* autonomous colonies (*An. aquasalis* and *An. albitarsis*), a procedure that contributes to the understanding of several aspects of their development and reproduction and that enables the use of those species as laboratory models. Basic parameters of development and reproduction of both species are being evaluated, such as the longevity of virgin and coupled females, blood and/or sugar fed, starving resistance and influence of the time of contact among males and females on copula effectiveness. We verified that *An aquasalis* are more resistant to starving than *An. albitarsis* and females are more resistant than males. The tax of inseminated females is directly proportional to the period of contact between males and females. *An aquasalis* longevity does not differ significantly between virgin and coupled females and coupled, blood and/or sugar fed Our results are being used in the optimization of *Anopheles* colonies maintenance, a prerequisite to the development of rational malaria vectors control strategies.

VE20 - Some approaches to study the *Culex quinquefasciatus* vitellogenesis

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Culex quinquefasciatus is a cosmopolitan, domestic, and highly anthropophilic mosquito. In order to improve our knowledge on the physiology of this animal, nocturnal bother and cause of allergies, we present here a preliminary characterization of the principal organs involved in its vitellogenesis. Trophocytes of the fat body are responsible for the synthesis of vitellogenin (Vg) which, carried by the hemolymph, is internalized by the developing oocytes as yolk protein. Around 12 h post blood meal, (PBM), Vg synthesis is characterized by the enlargement of rough endoplasmic reticulum (RER) and the formation of dense secretion vesicles in the Golgi complexes that continue until 48 h PBM. At this time, the degradation of the biosynthetic machinery by autophagosomes starts. Only 84 h PBM, the fat body returns to its pre blood meal morphology. The amount of hemolymph proteins during this period shows a peak at 48 h PBM and returning to basal values at 96 h PBM. Ovarian follicles of adult females before blood feeding contain a cluster of undifferentiated cells surrounded by a layer of follicular epithelium. After the blood meal, numerous microvilli on the oocyte membrane increase the uptake of Vg, which is stored together with abundant lipid inclusions. Between 24 and 48 h PBM is observed a marked increase of the ovarian total proteins, and no morphological alterations are seen in oocytes until 96 h PBM, when glycogen deposits are observed. Around 12 h PBM, follicular epithelium begin to develop RER. The space between follicular cells and oocyte starts to be filled with electrondense plates that will make the endochorion. Between 48 and 52 h PBM, electrondense vesicles fuse to form small granules in the apical membrane that, apparently, will originate the exochorion. AFC and RLC are FAPESP fellowships.

VE21 - Scanning electron microscopy study of the mosquito heart

Martins, G.F. *(Centro de Pesquisas René Rachou-Fiocruz)*; Pimenta, P.F.P. *(Centro de Pesquisas René Rachou-Fiocruz)*

The dorsal vessel is the main system in which the hemolymph is pumped into the insect. It is a pulsate organ that extends dorsally from the head to the end of the abdomen, and is divided into a posterior heart with perforations restricted to the abdomen, and an anterior imperforated aorta. In the present work, we describe the heart structure of some mosquito species by scanning electron microscopy. Males and females of the following species were used: *Aedes albopictus* , *Ae. aegypti* ,*Ae. fluviatilis*, *Culex quiquefasciatus*, *Anopheles darlingi* and *An. aquasalis*. The opened abdomen was fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2, post-fixed in 1% osmium tetroxide in 0.8% potassium ferrycianide, dehydrated in crescent series of acetone, dried in a critical point device using CO_2 and gold coated. The mosquito hearts were seen as a muscular tube, with alary muscles and pericardial cells along its sides. Pericardial cells are smooth and rounded and placed in the edges of the heart in single lines on each side of the organ. Alary muscles were attached to the body wall, extended to the dorsal vessel, and were inserted above the heart. Alary fibers presented a striated aspect and in the neighboring heart region they ramified, forming a network. These fibers were anastomosed above the heart surface and seemed to provide an attachment site for pericardial cells. The heart's openings, or ostia, represented the structure where hemolymph enters to be pumped into the heart. Sometimes the heart wall was broken, exposing the circular striated aspect of the interior face of the heart wall. Accordingly to our knowledge, this is the first SEM study of a mosquito heart. Further studies are being done in order to better understand the organ morphology and physiology. Financial support: Fapemig, Pronex-CNPq, Fiocruz and CAPES

VE22 - Primary culture of the oenocytes from the *Aedes aegytpi* fat body

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Fat body of insects is a multifunctional organ for storage and release of hemolymph components. The mosquito fat body is formed by two cell types: the trophocyte, responsible for the egg yolk precursor supply and the oenocyte that function is unknown. The main purpose of the present work is to establish the primary culture of the oenocytes from Aedes aegytpi fat body and to study morphological aspects of these cells in primary culture. Oenocytes were isolated from the fat body of *A. aegypti*, tripsinized in 1% tripsin and maintained in IPL41 culture media, supplemented with antibiotics, adhered in cover slips pretreated with poly-L-lisin. Adherent cells were stained by Giemsa and for viability essay; they were incubated with 0.01% acridine orange in PBS at pH 7.2 for 1h and analyzed in an epifluorescence microscope. Adherent cells, after one day in culture, were fixed in 4% paraformaldehyde in PBS and stained by Phaloidin/FITC for laser confocal microscopy. For electron scanning microscopy, cells were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2, post-fixed in 1% osmium tetroxide in 0.8% potassium ferrycianide, dehydrated in crescent series of acetone, dried in a critical point device using CO_2 and gold coated. The viability assay showed that 85% of the cells were viable until 57^{th} day of the experiment. Oenocytes are oval-shaped cells and with cell prolongations that correspond to filopodia and lamellipodia. The oenocytes cytoplasm showed vesicles that were not stained by Phalloidin. These structures may correspond to secretion granules or cell canaliculum system. Considering that oenocytes are enigmatic cells, which function is unknown, we believe that the primary cultivation can contribute for this question to be answered in the future. Financial support: Fapemig, Pronex-CNPq, Fiocruz and CAPES

VE23 - *Aedes* immune responses to viral infection

Nascimento-Silva, M.C.L. *(Universidade Federal do Rio de Janeiro)*; Oliveira, P.L. *(Universidade Federal do Rio de Janeiro)*; Sorgine, M.H.F. *(Universidade Federal do Rio de Janeiro)*

Diseases caused by arthropod-born viruses represent a significant public health problem, and novel control methods are needed to block these pathogens transmission. Although *Aedes aegypti* is the major vector of both yellow fever and dengue virus, little is known about mosquito responses to viral infection. The understanding of insect immunity has increased a lot in the last years, among other reasons for the release of insect genomes and the identification of major similarities between insect innate immune pathways and similar pathways from other organisms. Despite the enormous importance of understanding the innate immune system of disease vectors, little is known about mosquitoes immune pathways. In this work we used infections with Sindbis virus as model to understand virus-mosquitoes immune interactions. TIGR database were used to select two hundred immune related genes that had differential expression in dengue virus infect *A. aegypti* EST libraries when compared with other *A. aegypti* EST libraries. The genes were amplified by PCR using T3 and T7 vector primers and will be spotted onto a macroarray chip. mRNA of C6/36, infected or not with sindbis virus, will be used to probe the chip in order to identify genes differentially regulated by the infection. In addition, mosquitoes were artificially infected with Sindbis virus and the expression of some central immune genes was investigated by Real Time PCR. Rel1, Rel2 and STAT had their expression highly increased in infected mosquitoes when compared with non-infected animals. This approach will give us a better understanding of how the mosquitoes immune system respond to virus infection, allowing most specific investigations of each immune pathway and mediators.

VE24 - Egg morphometry and morphology of *Cimex lectularius* (Hemiptera, Cimicidae) Rosa, J.A. da (FCF/UNESP); Mendonça, V.J. (IB/UNICAMP); Miné, J.C. (FCF/UNESP); Pinto, M.C. (FCF/UNESP); Graminha, M.A.S.(FCF/UNESP); Justino(SSGP)

Rosa, J.A. da *(Faculdade de Ciências Farmacêuticas/UNESP/Araraquara)*; Mendonça, V.J. *(Instituto de Biologia/UNICAMP)*; Miné, J.C. *(Faculdade de Ciências Farmacêuticas/UNESP/Araraquara)*; Pinto, M.C. *(Faculdade de Ciências Farmacêuticas/UNESP/Araraquara)*; Graminha, M.A.S. *(Faculdade de Ciências Farmacêuticas/UNESP/Araraquara)*; Justino, H.H.G. *(Secretaria de Saúde de Gavião Peixoto)*

In the 31 of May, 2006, around 150 *Cimex lectularius* samples were collected in a 50 years old masonry home, located at Nova Paulicéia district, Gavião Peixoto City, São Paulo. Three bedroom mattress, a hall sofa and bedroom walls cracks were infested. The dwellers are four male, which does not use bed sheets over the mattress, neither clean the house, although they appeared to be healthy. In the walls, feces vestiges were perceived in several spots and many insect colonies with closely eight samples in each one of them. The samples are kept in a laboratory of Parasitology at FCF/UNESP in environment temperature, weekly fed with Swiss mice, albinos. Fifty ellipsoidal eggshells were measured through image analyzer, using the Leica Qwin program. Operculum opening diameter average: 0,22 mm, average length: 1,06 mm, average width: 0,41 mm. The eggshell exam by scanning electron microscope showed that the exochorion is formed by a structure set that resembles little blisters. These blisters gather

making polygon figures.

VE25 - The Effect of Anti Parasite Genes in Transgenic Mosquitoes

BURINI, B. C. *(Universidade de Sao Paulo)*; BONALDO, M. *(FUNDACAO OSVALDO CRUZ)*; CAPURRO, M. L. *(UNIVERSIDADE DE SAO PAULO)*

Malaria is one of the most deadliest infectious disease in the world. It kills about 3 million people every year, most of them in Africa. This disease is caused by plasmodia and is transmitted to humans by the bite of female Anopheles mosquito. The lack of an efficient vaccine combined with parasite drug resistance and mosquito insecticide resistance has brought about a necessity for a new measure to control this disease. Recent advances in genetic engineering make genetically modified mosquitoes that are incapable of transmitting the disease to humans a promising strategy for efficient control of malaria. Previous studies have suggested that the immunodominant circumsporozoite protein present on the surface of the plasmodia at the sporozoite stage could be responsible for penetration into the salivary glands of the mosquito. This is a crucial step in the life cycle of the parasite for transmission of the disease into a vertebrate host. The aim of this project is to express recombinant peptides for the conserved region I and Region II of the circumsporozoite protein in the mosquito hemolymph. We hypothesize that the recombinant Region I and II peptides will compete with the protein present on the surface of the sporozoites and prevent its penetration into the salivary glands. To choose the peptide which will result in an efficient blocking of this process, a transient expression system dsSindbis was used. These recombinant peptides are currently being tested for their efficacy. Recombinant peptides that efficiently blocks sporozoite penetration into salivary glands will be used to generate transgenic lines.

VE26 - VERTICAL TRANSMISSION OF DENGUE VIRUS BY ARTIFICIALLY INFECTED *Aedes aegypti*

Campanelli, E.S. *(Centro de Pesquisas René Rachou - FIOCRUZ/MG)*; Oliveira, A.C.S. *(Fundação Ezequiel Dias)*; Souza, K.P.R. *(Fundação Ezequiel Dias)*; Cecílio, A.B. *(Fundação Ezequiel Dias)*; Pimenta, P.F.P. *(Centro de Pesquisas René Rachou/ FIOCRUZ/MG)*

Dengue is a viral disease transmitted by mosquitoes and caused by four distinct viral serotypes (DENV 1-4). This mosquito-borne disease is a major public health problem and is a threat to more than 2.5 billion people, who live in endemic areas. *Aedes aegypti* is the primary vector of dengue in the Americas. After ingesting an infectious blood meal, the virus infects and replicates in the insect midgut, and then, when the virions are in the hemolymph, other organs of the mosquito can be infected, such as the salivary glands and the ovaries. If the ovary becomes infected, dengue virus can be transmitted to the progeny (vertical transmission), this mechanism is considered to be an important aspect of the maintenance of the virus during inter-epidemic periods. The aim of this study was to detect the presence of this mechanism of transmission in mosquitoes artificially infected with dengue virus. *A. aegypti* females from a laboratory colony were infected with DENV-2 using a membrane feeding technique. Three days post infection; engorged females were separated and forced to lay eggs. After the oviposition, females were frozen to be later analyzed by RT-PCR. At the same time, the eggs were placed in containers for the larvae to hatch. Then, when the larvae reached the 4th instar, they were separated, and frozen to be analyzed by RT-PCR. At this moment, parental females and descendant larvae were tested for the presence of dengue virus by RT-PCR. Our results showed the presence of the virus on the offsprings of the infected females. This proves that, not only the females of the colony tested have become infected, but that they are also able to transmit the virus to its progeny.
Support:FIOCRUZ, FAPEMIG, CNPq e PRONEX.

VE27 - Lipophorin from *Cimex hemipterus*: Purification and characterization.

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The cimicides, known vulgarly as bed chinch-bugs, are hemipterus hematophagous, in its majority are parasitic of bats, being able to parasite domestic birds, animals and the man. There are six families, but only two, Cimicidae (*Cimex hemipterus* and *Cimex lectularius*) and Cacodminae (*Leptocimex boueti*), possess species that parasites the man (Usinger, 1966). These insects come being considered as pathogens microoganisms transmitters (bacteria, fungi and virus), since they possess obligator hematophagy. Under the point of view of public health, the high tax of infestation of these insects can cause bother, sleepness, allergic reactions and anemia in the children population and domestic animals, when the infestation occurs in its shelters. They possess next nocturnal habits lodging its alimentary source. Lipid transport in arthropods is achieved by highly specialized lipoproteins, which resemble those described in vertebrate blood. Here, we describe purification and characterization of the lipid-apolipoprotein complex, lipophorin (Lp), in cimicides. Ten adults were fed on blood enriched with 3H-palmitic acid. One day later the bugs were homogenized and subjected to a KBr density ultracentrifugation. A lipid labeled lipoprotein was isolated from the top of gradient. This lipoprotein, resemble the typical lipoprotein responsible for lipid transport in insects hemolymph, also called lipophorin. The larvae lipophorin showed a hydrated density of 1.36 g/ml. SDS-PAGE revealed two apolipoproteins with mass similar

to those of apolipophorins (apoLp-I, 255 +/- 14 kDa; apoLp-II, 81 +/- 6 kDa).

VE28 - The antioxidant *Aedes aegypti* heme degradation product

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Blood digestion in the midgut of hematophagous animals results in the release of hemoglobin prosthetic group, heme, which is a pro-oxidant molecule. The enzymatic degradation of heme, that was already described in several organisms including plants, bacterias and mammals, is catalyzed by heme oxygenase and results in carbon monoxide, ferrous ion and biliverdin IX alpha. We have demonstrated that *Aedes aegypti* metabolizes heme into an alpha biliverdin isomer conjugated to two glutamine residues, thus generating a hydrosoluble bilin pigment. We proposed that *A. aegypti* biliverdin (AaBV) production pathway is composed from a heme degradation step, catalyzed by a heme oxygenase identified in the midgut epithelium, followed by two sequential additions of glutamine residues. We have observed by real time PCR that heme oxygenase expression in the midgut is upregulated by blood feeding, reaching maximum values between 24 and 42h after blood meal. In mammals heme is converted into biliverdin and subsequentially into bilirrubin, which is converted into a soluble glucuronic acid-conjugated form and excreted in the feces. Bile pigments were found to be potent antioxidants. Thereby we investigated the antioxidant capacity of AaBV. Here we show that it was able to prevent B-phycoerythrin oxidation by free radicals. This protection occurs in a dose dependent manner, as also observed for alpha-biliverdin isomer and trolox, well-known antioxidants. This antioxidant capacity was confirmed by western blotting against oxidized protein. Midgut epithelium homogenate was challenged with a free radical generation system in the presence or absence of different antioxidants, including AaBV. Once again AaBV revealed an antioxidant capacity comparable to other tested antioxidants. We believe that production of a hydrosoluble antioxidant from heme may represent an important mechanism to protect these hematophagous insects against oxidative damages promoted by heme. Support CNPq, CAPES, FAPERJ and PRONEX

VE29 - Ultrastructural Aspects and Carbohydrate Involvement in *Blastocrithidia culicis* - *Aedes aegypti* Salivary Gland Interaction

Nascimento, MTC *(Universidade Federal do Rio de Janeiro)*; Garcia, MCF *(Universidade Federal do Rio de Janeiro)*; Dias, FA *(Universidade Federal do Rio de Janeiro)*; Lopes, AH *(Universidade Federal do Rio de Janeiro)*; Motta, MCM *(Universidade Federal do Rio de Janeiro)*; Saraiva, EM *(Universidade Federal do Rio de Janeiro)*

We investigated *Blastocrithidia culicis* colonization of *Aedes aegypti*, an important vector of human diseases such as dengue and yellow fever, with the aim to study the development of monoxenic trypanosomatids in invertebrate host. Our group has already demonstrated that *B. culicis* is able to survive for a long time in the midgut of female mosquitoes and reach its haemocele (Correia-da-Silva et al, 2006). We were now interested in studing if that protozoan could adhere to the mosquito salivary gland. To address this question we incubated excised salivary glands of *A. aegypti* females with *B. culicis in vitro*. Our results demonstrated that this protozoan is able to bind to the salivary glands. Protozoa adhered to the salivary gland by introducing the flagella, as observed by scanning electron microscopy. To further investigate protozoa adhesion, we added different carbohydrates to the *B.culicis*-gland interaction medium. Our preliminary results showed that D-galactose, N-acetyl-D-glucosamine, alpha-methyl-D-mannoside and fucose inhibited this interaction. In order to characterize receptor(s) responsible for the protozoan recognition, we resolved total salivary gland proteins by SDS-PAGE followed by Western blot, identifying two bands of 28 and 31 KDa able to bind biotinylated-*B.culicis*. Western blots of total salivary gland proteins have also reacted with lectins. The bands that bound *B. culicis* were recognized by lectins of different carbohydrate specificities, evidencing their glycoprotein nature. Our results suggest the participation of carbohydrates in the *B.culicis*-*A. aegypti* salivary gland interaction. Supported by: CNPq, FAPERJ, CAPES, #F. A. Dias is recipient of FAPERJ Nota 10 fellowship

VE30 - Biochemical and molecular pyrethroid resistance mechanisms in one Brazilian *Aedes aegypti* population submitted to controlled selection pressure in the laboratory

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Resistance of Brazilian *Aedes aegypti* populations to the organophosphate temephos took 30 years to be first detected. In contrast, susceptibility alteration to pyrethroids was noted only 2-3 years after their initial use, in 2000-2001. In order to study, under controlled conditions, the insecticide resistance mechanisms selection dynamics, a field population, already resistant to both temephos and pyrethroid, is being submitted to pyrethroid selection through several generations in our laboratory (that is presently in conformity with biosafety rules). Two lineages are kept separately, each one in triplicate: "S"strain is maintained without insecticides and females of the "R"strain are selected with the pyrethroid

deltamethrin at each generation. Pyrethroids resistance, potencial cross resistance to temephos, as well as the mechanisms involved are being investigated. The latter include quantification of enzymes related to metabolic resistance and alterations of the pyrethroid target site (the sodium channel at the central nervous system). From F1 to F3, adults pyrethroid resistance increased in the R-strain. In the same period, activity of Acetylcholinesterase, the organophosphate target site, and of Mixed Function Oxidases remained unaltered. A slight decrease of Glutathione-S-transferase activity from F1 to F3 was noted in two out of three "R"replicas. By contrast, alfa-Esterase increased activity was noted in some individuals in two "R"replicas. A low frequency of altered individuals in a population is typical of incipient resistance. Analysis of mutations in the sodium channel of these strains is under way. It should be noted that Brazilian *A. aegypti* populations examined to date do not exhibit the classical kdr sodium channel mutation, that is associated with pyrethorid resistance. The results obtained with this procedure will increase knowledge of the insecticide resistance dynamics that occurs in Brazilian dengue vector populations and help the definition of rational control strategies.

VE31 - Acyl-CoA-binding protein gene expression in the midgut of *Rhodnius prolixus* does not depend on blood feeding

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Acyl-CoA esters have many functions in cells, such as energy source, substrate in metabolism and cell signaling. The acyl-CoA-binding protein (ACBP), a highly conserved 10 kDa intracellular protein, binds straight-chain long acyl-CoA esters with very high affinity, and protects acyl-CoA esters from hydrolysis, thus functioning as a reserve of these lipids by regulating their availability for a variety of metabolic purposes. In insects, the midgut epithelium cells absorb free fatty acids, and probably convert them to their CoA derivates prior to the synthesis of complex lipids, such as diacylglycerol (DG), that will then be transferred to hemolymphatic lipophorin, a major insect circulating lipoprotein. ACBP probably takes part in the process by transporting acyl-CoA to be used in these reactions. ACBP transcript has been sequenced from a *Rhodnius prolixus* midgut cDNA library and ClustalW alignment showed that it has great similarity with other ACBPs. Using RT-PCR, ACBP gene expression was detected in anterior and posterior midgut, fat body, ovary and flight muscle. ACBP gene expression was the analyzed by real-time PCR. Expression analysis of ACBP gene in the midgut showed a great increase in expression after blood meal, and it was very high from first to the fourth day after feeding and at seventh day it was similar to unfed levels in the seventh day. Insects fed only with Tyrode buffer also showed an great increase of ACBP expression. ACBP gene showed an expression increase of 80% in unfed female injected with hemolymph collected from insects fed 6 hours before. Injection of 15 pmol of Rosiglitazone, a PPARγ agonist, induced an increase of 75% of ACBP expression in unfed females when compared to control. This study intends to investigate the role of ACBP in intracellular lipid transport in *Rhodnius prolixus* midgut. Supported by CNPq, PIBIC/UFRJ and Faperj

VE32 - Effects of retinoids and terpenoids on *Rhodnius prolixus*: possible involvement of the *Ultraspiracle* (*RpUSP*) gene product

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Many of the highly prevalent tropical diseases in the world are transmited by hematophagous insects and one of the main strategies to reduce their spreading is based on the control of vector population through insecticides. Juvenile hormone (JH) analogs are being used as insecticides and this is based on the central role of JH in regulating many events such as embryogenesis, moult and immune response. It is believed that JH effects are mediated by a class of nuclear receptors named ultraspiracle (USP), which are orthologues of the retinoic acid receptors. Here we aimed to investigate the effects of retinoids and JH on the physiology of the insect *Rhodnius prolixus*. Injection of 9-cis retinoic acid (9cis RA) in the hemocoel of 4th instar nymphs led to remarkable changes in the external morphology upon moult, suggesting that it may be acting as a morphogen. Injection of retinoids and terpenoids decreased hemolymphatic phenoloxidase activity in adult insects. Moreover, we obtained a partial clone of the *R. prolixus USP (RpUSP)* of 879bp length, containing the two interaction domains of this protein. Analysis of the *in silico* translated sequence show that the predicted RpUSP protein is more similar to the orthologues from the *Locusta-Blattella-Melipona* group than to the *Amblyomma-Aedes-Drosophila* group. RT-PCR experiments reveled the presence of RpUSP in salivary glands, fat body and ovaries and a semi-quantitative RT-PCR indicated that this gene is more expressed in ovaries. Expression of RpUSP in fat bodies increased during blood digestion. Injection of 120 pmoles of 9cis RA or JH in the hemocoel of adult females increased RpUSP expression. Thus, the results presented here indicate that an authentic USP is coded in *R. prolixus* and possibly some of the physiological effects promoted by retinoids/terpenoids treatment may depend on RpUSP activity upon ligand binding. Financial support: CNPq, FUJB, Faperj, TWAS.

VE33 - Inhibitory effects of D-mannose in trypanosomatid lyses induced by *Serratia marcescens*

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Studies were carried out on the effects of different carbohydrates on the lysis of *Trypanosoma cruzi*, *Trypanosoma rangeli* and erythocytes caused by the bacteria *Serratia marcescens* variants SM 365 and RPH. High concentrations of D-mannose were found to protect *T. cruzi* and *T. rangeli* markedly diminishing the lyses caused by *S. marcescens*. However, this carbohydrate is unable to interfere with the hemolysis induced by SM 365 and RPH variants. These results showed that the trypanolytic effect induced by *S. marcescens* SM 365 and RPH variants is dependent of D-mannose and distinct from the hemolytic activity strongly suggesting that bacterial fimbriae are relevant to *S. marcescens* in lysis of parasites.

VE34 - A method to obtain trypanosomatid-free hemipteran insect *Oncopeltus fasciatus* (milkweed bug)

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The family Lygaeidae, order Hemiptera, constitutes the major group of phytophagous species that host lower trypanosomatids (21 species). Among them, 18 species are naturally infected with *Leptomonas* spp and 9 with *Phytomonas* spp. While the *Leptomonas* spp colonize only the digestive tract of their hosts, the Phytomonas spp trespass the intestinal epithelium, reaching the hemolymph and infecting the salivary glands. The lygaeid phytophagous *Oncopeltus fasciatus* is the natural host of *Phytomonas elmassiani*, *Crithidia acidophili*, *Leptomonas oncopelti* and *Leptomonas wallacei*. *O. fasciatus* has been described as a good model for studying host-parasite associations, both in natural and in experimental conditions. Trypanosomatid-free *O. fasciatus* insects could be considered as essential "tools" for studies aiming sound information about the interactions this hemipteran engages with every single species of trypanosomatids it harbors. In the present work eggs of *O. fasciatus*, obtained from a colony of insects naturally infected with *L. wallacei*, were treated with sodium hypochlorite and ethanol. Subsequently, parasite-free insects were obtained, as shown by means of optical and scanning electron microscopy, as well as by PCR. Supported by: CNPq, FAPERJ, CNPq/PIBIC-UFRJ, CAPES. F. A. Dias is recipient of "FAPERJ Nota 10" fellowship.

VE35 - Biochemical features of the cellular cysteine peptidases (cruzipain-like molecules) in Phytomonas serpens: implications on the parasite nutrition and interaction with the salivary glands explanted from the phytophagous insect Oncopeltus fasciatus

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Phytomonas serpens is a protozoan parasite assigned as a pathogen able to cause infection on different plants of great economic importance. In the present study, the cell-associated peptidase profile of *P. serpens* was evaluated. Promastigotes of *P. serpens* were cultivated in brain heart infusion supplemented with 10% fetal bovine serum for 48h at 26°C. Then, the cellular extracts were analyzed by gelatin-SDS-PAGE in order to characterize the biochemical features of the peptidases. The results showed the presence of two peptidases (38 and 40 kDa) that presented optimal activity at pH 5.0. The influence of different proteolytic inhibitors and reducing agents was also investigated. The peptidases were completely inhibited by E-64, leupeptin, cystatin, iodoacetamide and its catalytic activities were dependent on a reducing agent (e.g., dithiothreitol and 2-mercaptoethanol), characterizing them as cysteine peptidases. By means of Western blotting we demonstrated that the peptidases reacted with anti-cruzipain antibodies. Membrane extraction and immunocytochemical analyses revealed that the enzymes were located mainly in intracellular compartments, but could also be found on the parasite surface and on the flagellar pocket region. SDS-PAGE containing several proteinaceous compounds showed that the peptidases were capable of hydrolyzing casein, mucin, hemoglobin, immunoglobulin G, bovine serum albumin and extract of salivary glands of the phytophagous insect *Oncopeltus fasciatus*, suggesting that the cysteine peptidases have a wide spectrum of substrate cleavage. Additionally, the importance of these enzymes on the interaction of the parasite with the salivary glands of *O. fasciatus* was also studied. The association index between the flagellates and salivary glands was significantly reduced when the cysteine peptidases were blocked with specific cysteine proteolytic inhibitors or with anti-cruzipain antibodies. Collectively, these cysteine peptidases seem to participate in critical biological process of *P. serpens*, such as nutrition and interaction with the invertebrate host. Financial support: CNPq, FUJB, FAPERJ.

VE36 - Cellular Immune Response in =*Rhodnius prolixus*: Role of ecdysone in hemocyte phagocytosis

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In this work we investigate *in vivo* and *in vitro* effects of orally administered azadirachtin and ecdysone on the phagocytic responses of *Rhodnius prolixus* 5th-instar larval hemocytes to the yeast *Saccharomyces cerevisiae*. Groups of insects fed non-treated blood (control) and insects that received azadirachtin plus ecdysone in the blood meal were inoculated with yeast cells in the hemocele. The injected yeast cells disappeared rapidly from the hemolymph, being removed completely by 90 min after inoculation. In the insects treated only with azadirachtin the clearance of free yeast circulating particles was significantly delayed compared to the two previously mentioned groups.It was demonstrated that the binding of yeast cells to hemocytes was reduced in the insects treated only with azadirachtin in comparison to both non-treated control and azadirachtin plus ecdysone-treated groups. Phagocytosis occurred when yeast cells were added to hemocyte monolayers prepared with hemolymph from blood fed insects, treated or not with azadirachtin plus ecdysone, so that yeast cells were rapidly bound to hemocytes and internalized in high numbers. By contrast, insects treated with azadirachtin exhibited a drastic reduction in the quantity of yeast cell-hemocyte binding and subsequent internalization. In all groups, the hemocytes attached to the glass slides were predominantly plasmatocytes. The magnitude and speed of the cellular response suggests that hemocyte phagocytosis is one of the main driving forces for the clearance of free circulating yeast cells from the hemolymph. We propose that ecdysone modulates phagocytosis in *R. prolixus* larvae, and that this effect is antagonized by azadirachtin.

VE37 - Reactive Oxygen Species Production in the midgut of *Rhodnius prolixus*

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The hemipteran insect *Rhodnius prolixus* ingests large amount of vertebrate blood in a single meal and the hydrolysis of hemoglobin inside its digestive tract releases huge amounts of free heme, a pro-oxidant molecule that can iniciate an oxidative stress situation through an increase in the production of reactive oxygen species (ROS). Hematophagous insects deal with this situation by means of several antioxidant defenses. Besides the toxic effects of ROS it has been shown that these molecules have important physiological roles in a wide range of processes like cellular proliferation, inflammation and microbial killing. Little is known about the role of ROS in insect physiology, although a few recent papers have demonstrated that theses molecules might be involved in immunity towards pathogens. Here we studied the production of ROS in *Rhodnius prolixus* gut. Adult female insects were fed with rabbit blood and 3 days or 21 days after the blood meal (ABM) both anterior and posterior midguts were dissected and their contents were washed out with cold saline. Tissues were homogenized in PBS and centrifuged for 1 min at room temperature. Midgut epithelium hydrogen peroxide (H_2O_2) content was measured using scopoletin-horseradish peroxidase method. Three days ABM insects showed approximately 17,5 nmol of (H_2O_2) in the posterior midgut, compared to 4 nmol in the anterior midgut. The insects dissected 21-days ABM showed 2,6 nmol (H_2O_2) in the posterior midgut and 2,7 nmol in the anterior midgut. These findings suggest that the blood digestion inside the gut increase ROS production. The large pool of free heme that is created upon hemoglobin hydrolysis, potentially could interact with midgut-derived ROS, thus representing an extra source of oxidative stress. Financial support: CNPq, Faperj, FUJB.

VE38 - TRANSLOCATION OF TRIACYLGLYCROL LIPASE IN FAT BODY OF *RHODNIUS PROLIXUS*: A POSSIBLE REGULATORY MECHANISM

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In insects, the stored Triacylglycerol (TG) is an important reserve of lipids and its mobilization requires a triacylglycerol-lipase (TG-lipase) participation. In *Rhodnius prolixus* a TG-lipase activity was characterized in the fat body. Our results showed that the TG-lipase activity did not vary at different days after blood meal. This result suggested a mechanism similar to that observed in mammals, in which the TG-lipase is always present, but its activity is regulated by phosphorylation and translocation to lipid droplets. The study on the mechanisms involved in TG mobilization for energy production in non-mammalian species, particularly insects, may have important implications for the understanding of intracellular TG metabolism. The objective of this study is to investigate the distribution of TG-lipase activity in subcellular fractions on days after blood meal by Western blot analysis, using a rat hormone-sensitive lipase (HSL) antibody. Insects, at fourth, eighth, tenth and seventeenth days after blood meal were dissected and the fat bodies homogenized. The homogenates were fractionated by sucrose gradiente ultracentrifugation and analyzed using the anti-HSL antibody. The results showed that the antibody recognized a single band of 71 kDa, which change distribution along the gra-

dient according to the days after feeding. At fourth and seventeenth days, the bands were more intense towards the gradient bottom and at eighth and tenth days they were displaced towards the top. Lipid distribution in sucrose gradient fractions was analyzed by TLC, and TG was located at the top of gradients. These results suggested that translocation of the 71 kDa protein to the top of gradient was probably due to the association of the enzyme with lipid droplets, and this may be related with the previously described decrease in TG content in the fat body of *Rhodnius prolixus*, observed after thirteenth day after feeding. Supported by CNPq and FAPERJ.

VE39 - Morfometric Study of the Evolutional Instars of Four Colonies of *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera, Reduviidae)

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Lent & Wygodzinsky (1979) described 5 tribes, 14 genera and 112 Triatominae species. However, new genera and species had been described and currently 137 species are known (Galvão et al, 2006), amongst these *Triatoma rubrovaria*, that was of secondary importance, but probably due to environmental alterations come if domiciliating (Silveira, 1983). In this study, 15 units of each ninfal instar had been used of female and male adults and 50 eggshells of four colonies of *T. rubrovaria*, being three sylvatic habitat, collected in february/2002 in Alegrete and Quaraí (RS) cities and a Laboratorial colony, collected in april/1992 in Caçapava do Sul (RS); also they had been acording to the morphology of eggshells of the four colonies of *T. rubrovaria* by scanning electron microscope (SEM) and of three colonies of *T. rubrovaria* for transmission electron microscope (TEM). The morfometric study of the evolutional instar of head parameters (Dujardin et al, 1999), length thorax and abdominal showed that the Laboratorial and Lavras colonies are significantly bigger than the Quaraí and Quaraí 2. In the morfometric study of eggshells it was detected that opercular opening diameter as well as the relation length/width of the units of Laboratorial colony is significantly bigger than the others colonies.The scanning electron microscope (SEM) showed exocorial similar cells, differing in number of existing pores for cell in the units from Lavras, Quaraí and Quaraí2 colonies whereas the units of the Laboratorial colony is different in size and junctions between themselves. In the transmission electron microscope (TEM) was observed the existence of three exocoriais layers, distributed in similar way in the studied colonies. Supported by FAPESP, process number 03/13288-0

VE40 - Phylogeny and molecular taxonomy of *Triatoma sherlocki* Papa, Jurberg, Carcavallo, Cerqueira& Barata, 2002 and *T. brasiliensis* Neiva, 1911 (Hemiptera, Reduviidae) based on mithocondrial markers.

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Wild triatomine specimens collected at Santo Inacio - BA, 1975, were classified as *Triatoma brasiliensis* subspecie based on morphological and genetic studies. However, new analysis conducted at "Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos Fundação Oswaldo Cruz (FIOCRUZ)"in 2002 classified them as new species *Triatoma sherlocki* sp. n. Papa, Jurberg, Carcavallo, Cerqueira& Barata, 2002. In order to understand the phylogenetic relationship between *T. sherlocki* and *T. brasiliensis* (collected at Piauí and maintained at Faculdade de Saúde Pública/USP) we are currently investigating some regions of the the cytochrome b (mtCytB) and the large subunit ribosomal RNA (mtlsurRNA) mitochondrial genes. A 399-bp nucleotide sequence of the forward and reverse strands was determined for each examined species and analysed for homology to sequences in Genbank database. Phylogenetic analysis was performed by MEGA 3.1 using algorithm Neighbor-Joining (NJ) based on p-distance and a Kimura 2- matrix with bootstrap of 1000 replications. Pairwise identity comparison at the amino acid (aa) and nucleotide (nt) level of mtCytB sequences reveal identity between *T. sherlocki* and *T. brasiliensis* (the obtained sequence is similar to T. brasiliensis brasiliensis from Ceará) of 97% and 88%, respectively. Besides, *T. sherlocki* presents a closer affinity with the *T. brasiliensis melanica* from North of Minas Gerais indicated by an average identity between these two species of approximately 99% (aa) and 91% (nt). Despite morphological analysis showed *T. sherlocki* and *T. brasiliensis* as distinct taxa, our molecular data allow us infer a possible phylogeography based on *T. brasiliensis* ancestors. Supported: FAPESP (05/52608-6), FUNDUNESP (066/06) and CAPES.

VE41 - Further analysis of Lipid Rafts from *Rhodnius prolixus* midgut

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Rhodnius prolixus is a hemipteran haematophagous of the family Reduviidae, popularly known as ′kissing bug′. It doesn′t have preferential host, they feeds in any vertebrate of hot blood, besides the man. This insect is intermediate host of tripanossomatidaes, and pointed as principal vector of Chagas′s disease in countries as Venezuela and Colombia. The importance and the physiological role of midgut in lipid metabolism has been studied in different insect species. In

Rhodnius the midgut is able to transfer lipids to the main hemolymphatic lipoprotein, the lipophorin. Lipid rafts are microdomains detergent-resistant at low temperatures, composed of sphingolipids and cholesterol. They are involved in a number of cellular processes such a endocytosis, trafficking and cell signaling. The objective of this work is the purification and characterization of lipid rafts to define their role on lipophorin lipid transfer, and in the adhesion and invasion of tripanossomatidaes in *Rhodnius*. Sucrose density centrifugation is the standard method for lipid rafts isolation. One hundred *Rhodnius prolixus* were dissected, the midguts were removed, lysed, homogenized and incubated at 4^{o}C in 1% Triton X-100. After 30 minutes the homogenate was subjected to a sucrose gradient. The lipid raft was localized in fractions 3, 4, 5 and 6 of sucrose density gradient. Cholera toxin B subunit (CTB) is a specific ligand for ganglioside GM1 and can be used for the detection of GM1 containing microdomains. In order to investigate the presence of GM1, the fractions were submitted to a dot-blot using cholera toxin. The fractions also were subjected to a dot-blot using anti-caveolin, for to analyse the presence of caveolae. The fractions were subjected to lipid extraction and scraped from the high performance thin-layer chromatography (HPTLC). The spots were analysed by densitometry. Supported by CNPq, FAPERJ, IFS

VE42 - Wings morphometry and morphology of *Rhodnius neglectus* Lent, 1954 ,*Rhodnius pictipes* Stal, 1872 and *Triatoma infestans* (Klug, 1834) (Hemiptera,Reduviidae) Garcia A.N.(FCF/UNESP); Rosa, J. A. da (FCF/UNESP)

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The Chagas disease or American tripanossomiase was described in 1909, when Carlos Chagas was carrying out a campaing against malaria. Nowadays 137 Triatominae species are known and all of them can transmit *T. cruzi*. This present study measured the fore and hind wings of 15 samples of each species and sex, using image analysis and Leica Qwin software. Two colonies of each species were studied. The bugs were provided by Triatominae Insetary of Faculdade de Saúde Pública da Universidade de São Paulo (FCF/USP), placed in Serviço Especial de Saúde de Araraquara (SESA). This study has showed that there wasn′t significant difference between the right and left fore wings, as well as the right and left hind wings, of each colony in the three species, except in the CTA 057 *R. pictipes* colony, in which there was little significant difference between the female fore wings. The female average values for the fore and hind wings were bigger than the male ones. The male and female hind wings have area values smaller than the fore wings in all the studied species. In the morphologic study the transversal cut analysis of the Sc vein of the wings in three species was made by optic microscopy and transmission eletronic microscopy (TEM). The TEM analysis showed that the Sc vein of *R. neglectus, R. pictipes* and *T. infestans* is made up of a light surrounded by many tissue layers. The presence of a striated layer in the external portion of the structural tissue was observed in the Sc vein cut wing of *R. pictipes* and *T. infestans*. The presence of two paralel ducts to the light vein was observed in the Sc vein cut wing of *R. neglectus*. Supported by FAPESP, process 05/56780-8.

VE43 - ERK activation by stretch in *Rhodnius prolixus* midgut

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Blood-sucking arthropods are disease vectors worldwide. Despite the role of arthropod-blood feeding on pathogen acquisition and spread, the molecular mechanisms by which a blood meal is handled by such organisms remain unknown. *Rhodnius prolixus*, the vector of Chagas disease, ingests a huge amount of blood. Its digestive tract is divided in foregut, anterior midgut, posterior midgut and hindgut. The effect of anterior midgut stretch on the activation of intracellular signaling pathways is under investigation in our laboratory. We first evaluated mitogen activated protein kinase (MAPK) pathway, using antibodies against total ERK (extracellular-regulated kinase) or phosphorylated/active ERK (pERK). Our results show that anterior midgut stretch activates ERK. Such activation disappears twenty four hours after feeding insects with an artificial meal. ERK is phosphorylated/activated by MEK since insects fed with classic MEK inhibitors failed to phosphorylate ERK. This data corroborates the role of this MAPK pathway on this event. Identification of MEK activators is underway but they do not involve the uptake of extracellular calcium. Finally, the role of diuretic hormones and the intracellular proteins phosphorylated by MAPK pathway will be next evaluated. Supported by CNPq, FAPERJ, IFS and WHO.

VE44 - TRIATOMA INFESTANS CHOOSES THE IMMUNE PREY FOR FEEDING UPON

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Blood feeding *Triatoma infestans* obtained its fills from immune chickens in 15 min, but it needed 40 min for feeding upon non-immune chickens. High titer specific IgGs and skin reactivity against *T. infestans* saliva antigens were elicited in

immune chickens. Fluorescence-labeled leukocytes from non-immune or immune chickens were used to determine sources of blood drawn by equal numbers of triatomines distributed in separate compartments of a hut like box. It was shown that 64.4±4.7% of the reduviids were captured in the immune chicken room; 35.6±4.5% were present in the non-immune chicken dwell, and these differences were statistically significant ($p < 0.001$). Furthermore, *T. infestans* feeding upon immune birds reached the adult stage 40 days before those feeding upon non-immune birds, and differences were statistically significant. These results appear to have a broad epidemiological significance as for spreading enzootics; hence the immunological status of vertebrate host populations appears to favor *T. infestans* main transmitter of *Trypanosoma cruzi*.

VE45 - ALTERATIONS ON MAMMALIAN HOST SKIN MICROCIRCIRCULATION RESULTING FROM TRIATOMINES FEEDING PROCESS

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Triatominae bugs are obligatory haematophagous insects, taking blood from microvessels. Parameters of feeding behaviour are influenced by intrinsic characteristics of the feeding apparatus, such as size of the cibarial pump and by host factors related to haemostasis, such as probing time, frequency of cibarial pump contractions and interruptions during the engorgement phase. These parameters seem to act together, having a significant impact in the triatomines feeding performance. This work evaluated some aspects related to triatomines feeding process on mammalian hosts skin, such as choice of vessel (arteriole or venule), microvascular changes, leukocytes response and platelet aggregation. Intravital microscopy technique was used in order to access ear microcirculation of hairless mouse. During insect feeding process (*R. prolixus* and *T. infestans* nymphs), haemorrhagic regions in the skin were frequently observed (> 90%), occurring mostly during the probing phase. Using vital labels , it was observed an increase of vascular permeability after insects probing in all experiments. Both species introduced their maxillae preferable in venules than in arterioles. However, when comparing engorgement time either in arteriole or venule, arterioles seem to offer a more satisfactory environment for the feeding process. It was possible to evaluate vascular changes induced by both species. Venules used for *R. prolixus* feeding presented vasodilation (50%), whereas venules used for *T. infestans* feeding presented vasoconstriction (67%). It was observed a significant increase of rolling and adherent leukocytes after feeding of *R. prolixus* on mouse kin, characterizing an inflammatory process. In these experiments, it was also visualised platelet adhesion and aggregation to the injured venular wall. The insect feeding process consists in a complex mechanism, in which both mechanical characteristics (mouthparts movement or vessels deformation due to the cibarial pump functioning) and saliva deposition act all together in the host microcirculation.

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VE46 - GIPL-Mediated expression of nitric oxide synthase in *Rhodnius prolixus* salivary glands

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Rhodnius prolixus is a blood-sucking bug whose saliva contains several bioactive compounds. Among them nitric oxide (NO) bound to a set of proteins named nitrophorins (NPs). It is an unique molecule used by the bug to feed on blood due to its powerful vasodilatory activity. NO is produced by a salivary gland nitric oxide synthase (NOS) but its production occurs in a different timespan of NPs. We have previously shown that NP synthesis during the fourth to the fifth instar nymphs is under direct control of the protein kinase CK2 (Mesquita et al. 2005). We are now focusing on the mechanisms underlying NOS regulation. NOS activity increases significantly only after insect moulting i.e. 10-15 days following a blood meal. Using semi-quantitative RT-PCR and western blotting analysis we observed that the regulation of NOS in our model is performed at the transcriptional level. NP loading with NO is strongly decreased by *Trypanosoma rangeli* infection on salivary glands as shown by experiments with the NO fluorescent probe (DAF- Molecular Probes). Immunolocalization procedures using anti-NOS antibodies confirmed that *T. rangeli* decreased NOS levels in infected glands. In order to elucidate the mechanism utilized by the parasite to subvert NOS expression, we have injected parasite-derived surface glycoinositolphospholipids (GIPL) in *Rhodnius* nymphs. *T. rangeli* GIPLs reduced both NOS expression and activity. In spite of this, the injection of GIPLs derived from other protozoans, such as *Phytomonas serpens* and *P. françai*, did not significantly interfere in NOS activity. This data suggests that *T. rangeli* GIPLs is at least partially responsible for the manipulation of *Rhodnius* NOS expression during infection. The intracellular signaling pathway triggered by GIPL on salivary glands remains to be demonstrated. Such understanding might shed light on the mechanisms used by the insect to keep NOS activity synchronized with NP synthesis.

VE47 - Apyrase anti-haemostatic activity in the salivary gland of *Rhodnius spp*

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Blood-feeding arthropods are able to constraints barriers imposed by host defenses, due to the presence of a wide range of antihemostatic factors in their saliva, including vasodilators, antiplatelet factors and anticoagulant. In this work, we identified for the first time apyrase activities in the saliva of *Rhodnius brethesi*, *Rhodnius milesi*, *Rhodnius pictipes* e *Rhodnius robustus*. Enzymatic assays performed at 37 ^{o}C and pH 8.3, revealed that these apyrase activities are dependent upon $Ca^{+}2$. The apyrase activities present in the salivary glands of *R. brethesi* and *R. pictipes* were then partially purified on Oligo dT Cellulose® column. To identify proteins related to apyrase activity, salivary gland content and eluted fractions from the Oligo dT column were submitted to SDS-PAGE enzymography without previous boiling or reduction of the samples. This experiment allowed the identification of 44-45 kDa protein bands displaying both ATPase and ADPase activities. *In vitro* platelet aggregation assays showed that the content of 0.5 salivary gland pair *R. brethes*i, *R. milesi*, *R. pictipes* and *R. robustus* completely abolished platelets aggregation induced by ADP. The wide distribution of apyrases in the saliva of Chagas disease vectors and other arthropods indicates that these enzymes play an important role during the hematophagous process. Supported by Capes and CNPq

VE48 - HEMOCYTES FROM UNINFECTED TRIATOME AS TARGET CELL IN COMPLEMENT MEDIATED LYSIS.

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Immunological relationship between hemocytes (Hc) from uninfected *Trypanosoma cruzi* hematophagous vectors and infected mammal hosts was investigated. We reported previously that sera from Chagas disease patients recognize Hc from several triatomine species. Further, mice immunized with Hc, when challenged with *T.cruzi*, presented lower parasitemia than the control group. The ongoing parasitemia in chagasic infection is detected by the complement mediated lysis (CoML) technique, which uses living trypomastigotes and sera from infected individuals. Since Hc and trypomastigotes share common epitopes, we performed this technique exchanging the target, trypanosomes for Hc. Sera from *Calomys callosus* infected with a silvatic strain of *T.cruzi* were assayed. Sera were collected before the infection (N), at the ascendent phase of parasitemia (A), at the peak (P) and at the chronic phase (C). Trypanosomes were obtained from immunosupressed mice and Hc from *P.megistus* 5th instar nymphs. Each well from a polystirene microplate received 50μL serum dilution (1/4) in Eagle medium supplemented with 10% calf sera plus 100 μL of a trypomastigote suspension, containing 5 x 10^{6} parasites/mL or 2 x 10^{6} Hc. After 30 min at 37^{0}C, plates were kept on ice, the moisture divided in two samples and 50 μL human serum were added as source of complement. Total number of parasites was counted immediately and after 60 min. Lysis was expressed in percentage, as the average of two wells. The results showed: N=11/17; A=37/33; P=42/49 and C=29/32 (% trypomastigotes lysis/% Hc lysis). Unlike the conventional serological tests performed with epimastigotes, no cross-reativity between chagasic patient sera and leishmaniasis patient sera was observed in the CoML performed with Hc. All the sera presented negative results, i.e., their values ranged from 0 to 20%. The data obtained here corroborate our previous findings that Hc and trypomastigotes share common epitopes.