BQ001 - **FUNCTIONAL CHARACTERIZATION OF HISTIDINE METABOLISM IN *TRYPANOSOMA CRUZI***

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Trypanosomatids are able to catabolize amino acids. Moreover, it has been reported that amino acids have non-canonical roles on the biology of *T. cruzi*. Histidine is one of the most abundant amino acid in the hemolymph and excreted fluids of the insect vector. Due to the high exposition of *T. cruzi* to this metabolite we characterized its uptake and metabolism. His is incorporated trough a specific and active transport system, and processed by two enzymes: histidine ammonia-lyase (*Tc*HAL) and urocanate hydratase (*Tc*UH), rendering 4-imidazolone-5-propionate, which can be converted in α-ketoglutarate in a non-enzymatic way. In the present work we evaluated the functional role of this pathway in *T. cruzi*. We observed 75% more activity of His uptake in epimastigotes than metacyclic trypomastigotes. This fact, together with a higher expression of *Tc*HAL and *Tc*UH in epimastigotes, suggests a metabolism with a higher dependence on His in this stage. To evaluate the role of His as energy and carbon source in epimastigotes, we measured the oxidative catabolism of L-[^{14}C(U)]-His to $^{14}CO_2$. It was observed that 30% of the radioactivity incorporated with His was recovered as $K_2{}^{14}CO_3$ in 1 h, showing that His can be considered as an important oxidable source. Furthermore, when a saturable concentration of glucose was added, the $^{14}CO_2$ production diminishes approximately 30%, showing that His can replace glucose as an energy source. Also, we measured the ATP production of epimastigotes in PBS with a saturable His concentration. We showed that His is responsible for 50% of the ATP produced when parasites are incubated in LIT. The role of enzymes linking histidine metabolism with NADPH and low molecular weight thiols were also studied in H_2O_2 challenged or not parasites. No differences were observed, suggesting a more complex mechanism involved in parasite resistance to H_2O_2. Our data show that His has a main role in the energetic metabolism in *T. cruzi*.. **Supported by:**FAPESP

BQ002 - **INDUCTION OF RESISTANCE TO INHIBITORS OF STEROL BIOSYNTHESIS ALTERS THE GENE EXPRESSION OF THIS PATHWAY AND THE UPTAKE OF EXOGENOUS CHOLESTEROL IN *L. AMAZONENSIS* AND *L. BRAZILIENSIS***

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The ergosterol biosynthesis inhibitors, simvastatin, terbinafine and miconazole have shown in vitro activity on *L. braziliensis* and *L. amazonensis*. Despite the mechanism of action of these agents is well known, the leishmanial resistance has not been aimed yet. This work aims to study the mechanism of resistance of these inhibitors, evaluating the modulation of enzymes of the sterol biosynthesis pathway and the use of exogenous cholesterol by parasites. *L. amazonensis* and *L. braziliensis* promastigotes were grown with increasing concentrations of simvastatin, terbinafine and miconazole. The drug pressure initiated with the individual IC_{50} of each inhibitor and the concentration was increased step by step. These inhibitors (simvastitin, terbinafine and miconazole) showed a index of resistance of 3, 3 and 8 fold on *L. amazonensis* and 3, 2, 6 fold on *L. braziliensis* promastigotes, respectively. The cross resistance was also evaluated, with these inhibitors and reference drugs (miltefosine, amphotericin B and antimony III). The evaluation of sterol biosynthesis pathway was made by studying the expression of enzymes of this pathway (HMGCoA, esqualeno sintetase, esqueleno epoxidase, lanosterol sintetase, lanosterol C-14 demethylase and sterol metiltransferase) by real time PCR. The enzymes HMGCoA and C-14 demethylase are more modulated than other enzymes, independent of the used drug, varying of up or down regulation in the resistant strains. The sterol biosynthesis was evaluated by thin-layer chromatography (TLC). *L. amazonensis* and *L. braziliensis* promastigotes resistant to miconazole and terbinafine showed alteration of sterol biosynthesis and increased the uptake of exogenous cholesterol. Taken together, these results suggest that sterol biosynthesis pathway is dynamic in altering expression of enzymes as mechanism of resistance and moreover, the increase of cholesterol content may suggest a substitution of ergosterol as a compensatory mechanism. **Supported by:**CNPq

BQ003 - **CHARACTERIZATION OF OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* INOSITOL PHOSPHOCERAMIDE**

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Leishmania (Viannia) braziliensis is a widespread parasite in Brazil and the causative agent of cutaneous leishmaniasis, and occasionally mucosal or mucocutaneous diseases. Leishmania in general expresses a characteristic class of sphingolipid, the inositol phosphoceramide (IPC). The main IPC specie described in *Leishmania (Leishmania) major* and *Leishmania (Leishmania) amazonensis* presents a characteristic peak at *m/z* 778 represented by ceramide d36:1, containing 16:1 sphingosine and 18:0 fatty acid (Hsu et al. 2008, and Godoy et al. 2011). In this study we isolated IPC from promastigote of *L. braziliensis* (MHOM/BR/1987/M11272 and MHOM/BR/1975/M2903 strains) and analyzed by electrospray ionization-mass spectrometry using positive and negative ion modes. It was identified IPC species at *m/z* 752 (d32:0), *m/z* 778 (d34:1), *m/z* 780 (d34:0), *m/z* 806 (d36:1), and *m/z* 808 (d36:0). Two prominent ion peaks were detected at *m/z* 778.4 and *m/z* 780.4. Their structures were determined as d20:1/14:0 and d20:0/14:0 for both strains, which clearly a differ from IPC isolated from *L. major* and *L. amazonensis*, which present d16:1/18:0 and d16:0/18:0, suggesting expression of sphingosine synthases with different specificities, as serine stearoyltransferase for *L. braziliensis*, and serine myristoyltransferase for *L. major* and *L. amazonensis*. These results indicate that *L. braziliensis* expresses IPC with a longer sphingosine compared to other Leishmania species. At present we are performing a comparison of IPC species found in *Viannia* subgennus: *Leishmania (V.) naiffi, Leishmania (V.) guyanensis and Leishmania (V.) pananmensis*. These results may contribute to better understand parasite IPC biological roles and to identify new targets for chemotherapies against leishmaniasis. **Supported by:**FAPESP, CNPq and CAPES

BQ004 - **HEME STIMULATES NA+/K+ ATPASE ACTIVITY TROUGH HYDROGEN PEROXIDE GENERATION IN *LEISHMANIA AMAZONENSIS***

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Leishmania amazonensis is a protozoan that occurs in many areas of Brazil and causes cutaneous lesions. A recent work of our group has shown the activation of a Na^+/K^+ ATPase in *L. amazonensis*, through a signal transduction cascade involving the presence of heme and PKC activity. Heme is an important biomolecule with a pro-oxidant and signaling capacity. Recently, hydrogen peroxide (H_2O_2) has been considered an important second messenger, being able to stimulate PKC activity in several models. Our goal in this work is to investigate the role of heme-dependent hydrogen peroxide generation on Na^+/K^+ ATPase activity of *L. amazonensis*. Our results shows that increased concentrations of heme, stimulated H_2O_2 generation in a dose dependent manner, reaching its maximum at 2,5 µM. We also tested the effect of protoporphyrin IX, a precursor of heme, Co_protoporphyrin, Sn_protoporphyrin, and the products of heme degradation, bilirubin, and biliverdin on H_2O_2 generation, and none of them caused any effect. We evaluated Na^+/K^+ ATPase activity using increasing concentration of H_2O_2. As expected, Na^+/K^+ ATPase was stimulated by increasing concentrations of this reactant and reached its maximum at 0.1 µM. In order to investigate the role of PKC in this signaling pathway, we verified the production of H_2O_2 in the presence of its activator phorbol 12-myristate 13-acetate (PMA) and inhibitor, calphostin C. Both had no effect on the generation of H2O2. Furthermore, we found that PKC activity is increased in the presence of H_2O_2 and in the presence of calphostin C, H_2O_2 is unable to activate the Na^+/K^+ ATPase. Thus, the results suggest that PKC is activated by H_2O_2 generated in the presence of heme, thus activating the Na^+/K^+ ATPase. We are now investigating where this H_2O_2 is produced and how heme interacts with the membrane bound of L. amazonensis (through a receptor or transporter). **Supported by:**CNPQ, FAPERJ, CAPES

BQ005 - **ENDOSYMBIOSIS IN TRYPANOSOMATIDS: THE ASSOCIATION BETWEEN THE BACTERIUM AND ENERGETIC ORGANELLES IS REVEALEAD BY ULTRASTRUCTURAL AND BIOCHEMICAL APPROCHES**

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Strigomonas culicis is a trypanosomatid that co-evolves in a mutualist relationship with a symbiotic bacterium and constitutes an excellent model to study origin of organelles and cell evolution. An interesting aspect of this relationship is the intense metabolic exchange between partners. This study aimed to investigate the association between symbiont and energetic organelles, glycosomes and mitochondrion, considering ultrastructural and biochemical aspects. Thus, different microscopy approaches were used to observe the ultraestructure of wild and aposymbiotic strains of *S. culicis*, as confocal microscopy, transmission electron microscopy (MET) and 3D reconstruction using electron tomography, as well as high resolution respirometry. Immunofluorescence assays revealed great proximity between symbiont and glycosomes, since glycosomes formed clusters around the bacterium. In the aposymbiotic strain glycosomes were found randomically distributed in the cytoplasm, either in the anterior or in the posterior part of the cell body. The close association between glycosomes and the symbiont was confirmed by MET and electron tomography, since the bacterium envelope was seen juxtaposed to the glycosome membrane. Such association was not observed between symbiont and mitochondrion. Regarding biochemical studies, we investigated the O_2 consumption in symbiont containing and aposymbiotic strains by testing different inhibitors of the electron transfer system as rotenone, oligomycin and potassium cyanide, we also added a mitochondrion uncoupler (FCCP). Interestingly, our results showed that symbiont containing cells consume three times more O_2 when compared to the symbiont free protozoa and responses facing different inhibitors were also distinct between both strains. Altogether, our data suggest that symbiont enhances host O_2 consumption. Furthermore, the glycosome-symbiont interaction supports the idea that bacterium benefits from host glycosome energy production. **Supported by:**INBEB, CAPES, CNPq e FAPERJ

BQ006 - **EVALUATION OF THE ROLE OF CK2 ON THE PROCESS OF INTERACTION BETWEEN *LEISHMANIA DONOVANI* AND MACROPHAGES USING 4,5,6,7-TETRABROMO-1H-BENZOTRIAZOLE (TBB) AS AN INHIBITOR**

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Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania*. When parasites invade a human host, they undergo metacyclogenesis, which generates their ability to interact with host cells. This interaction activates signal transduction pathways in both, host cells and parasites, which induce numerous biological mechanisms including protein kinase 2 (CK2). This enzyme is capable of phosphorylating serine/threonine residues in their target proteins that participate in cell division, cell differentiation and DNA repair processes. It also has anti-apoptotic functions. Previously, CK2 has been described as an important enzyme in the interaction process between mammalian cells and intracellular parasites such as *Leishmania* and *Trypanosoma* species. In this work, we have studied the participation of CK2 in the biology of *Leishmania donovani*, including promastigote- and amastigote-macrophage interactions using TBB, a highly selective inhibitor of CK2 kinase activity. It acts as an ATP competitor that binds in the catalytic site of CK2. The application of TBB was capable of inhibiting the growth of *Leishmania donovani* promastigotes by approximately 25%. However, this effect did not appear to occur by apoptosis since this form of the parasites did not externalize phosphatidylserine in comparison to a treatment with miltefosine, which served as a positive control. In the case of interactions, TBB promoted a significant reduction in the number of amastigotes measured inside infected cells. Nitric oxide production was not altered by the use of TBB. However, this effect on amastigotes surveillance could possibly occur through apoptosis as demonstrated by the TUNEL technique and considering the anti-apoptotic effect of CK2. The viability of macrophages was not been affected by the concentrations of TBB used in this work. The results continue to support the important role of CK2 in the process of *L. donovani* interacting with macrophages. In addition, TBB appears to have a leishmanicidal role without affecting the viability of the host cells. Continued studies on CK2 and its inhibitors should prove helpful in the development of new drugs for the treatment of Leishmaniasis. **Supported by:**CNPq and Faperj

BQ007 - ***TRYPANOSOMA CRUZI* : NITRIC OXIDE SIGNALING DUE TO PARASITE INTERACTION WITH THE EXTRACELLULAR MATRIX.**

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Nitric oxide (NO) is a second messenger biosynthesized from L-Arginine and involved in cell signaling by different mechanisms: activation of cGMP production by guanilyl cyclase; regulation of enzymes by interaction with their metallic centers; or by S-nitrosylation of cysteine, a posttranslational modification capable of modulating several proteins. In *Trypanosoma cruzi*, the etiological agent of Chagas disease, very little is known regarding NO signaling, although NO synthase (NOS) activity has been previously reported (Paveto C et al., 1995, J. Biol. Chem. 270: 16576–79*)* and S-nitrosylation targets were identified using NO donors (Venturini G *et al.,* 2000, Biochem Biophys Res Commun 270: 437–41*)*. Since the interaction between *T. cruzi* and the host extracellular matrix (ECM) triggers signaling events such as protein phosphorylation (Mattos EC et al., 2012, PloS One 7: e46767), we decided to investigate whether this interaction could also modulate NO signaling in *T. cruzi*. Parasites were incubated with ECM or bovine serum albumin and NO and cGMP production, NOS activity and the profile of S-nitrosylated proteins were compared. It was found that NOS activity, NO and cGMP production were markedly decreased after incubation of *T. cruzi* with ECM. Accordingly, a decrease in protein S-nitrosylation in p-formaldehyde-fixed parasites was also observed, as evidenced by immunofluorescence using anti-NOCys antibodies. In addition, the S-nitrosylated protein profile was modified in ECM-incubated parasites. For instance, dual specificity protein phosphatase and serine/threonine protein kinase were denitrosylated upon incubation with ECM, whereas no changes in S-nitrosylation of surface protease gp63 was found upon incubation with ECM. Taken together, the results suggest that NO metabolism is modulated during adhesion of *T. cruzi* to components of the extracellular matrix, probably by the classical cGMP pathway and S-nitrosylation.**Supported by:**FAPESP

BQ008 - **IDENTIFICATION OF IMMUNOGENIC PROTEINS OF *TRYPANOSOMA CRUZI* BLOODSTREAM TRYPOMASTIGOTES**

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Chagas disease is endemic in Latin America and is caused by the protozoan *Trypanosoma cruzi.* The incidence of this illness increases mainly due to the immigration of latin americans to non-endemic areas. The clinical evolution of this disease could lead to cardiac or digestive failure and the available treatment is based on benznidazol, however its efficacy depends directly on early diagnosis. In acute phase, the diagnosis is done mainly by microscopy, while in the chronic phase usually is performed by serologic tests. However, there is a lack of rapid tests that could be used in work fields or blood bank emergency. In this context, we revealed immunereactive proteins from *T. cruzi* bloodstream trypomastigotes (Y strain) using infected mice serum (22° dpi) and subsequently identify by mass spectrometry. After the padronization of bidimensional electrophoresis technique, the Western Blot was carried out and resulted in 62 selected spots reproductible among independent biological samples, which 13 parasite polypeptidic chains were identified. Functional and localization analysis showed that the major part of *T. cruzi* identified proteins were cytoplasmic and participate in energetic metabolism. The evaluation of local alignment and the prediction of linear epitopes of lymphocytes B demonstrate the existence of some distinct immunogenic epitopes in the identified sequences, however due to the high similarity to *T. brucei* e *Leishmania* sp. observed, phosphoenolpyruvate carboxykinase, trypanothione reductase, arginine kinase, lipoamide dehydrogenase, TcC31.26, pyruvate phosphate dikinase, enolase, nucleoside phosphorilase, s-adenosyl homocysteine, phosphoglycerate kinase, stress induced protein, subunit A of vacuolar ATPase and heat shock-*like* 85 kDa are not promising candidates for the development of diagnostic kits. Thereby, it is necessary a careful and deep evaluation about the potential of these identified proteins for immunechromatographic tests. **Supported by:**CNPq, FAPERJ and FIOCRUZ

BQ009 - **PEPTIDASES OF *TRYPANOSOMA CRUZI* AND *RHODNIUS PROLIXUS*: A FASCINATING INTERFACE**

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The most abundant surface peptidase of *Leishmania* spp., gp63, is one of the best characterized metallopeptidase in the trypanosomatidae family. The etiological agent of Chagas' disease, *T. cruzi*, expresses multiple isoforms of the gp63 family. This enzyme is required for parasite infectivity in mammalian cells; however, its role in parasite-vector interaction has not been explored so far. Here, we have analyzed the effects of the treatment of *T. cruzi* with metallopeptidase inhibitors and anti-gp63 antibodies on the parasite adhesion to *Rhodnius prolixus* posterior midgut *ex vivo*. In parallel, we have compared the proteolytic profile of expressed peptidases in the insect vector. Briefly, the parasites were treated with 1,10-phenanthroline, EDTA and EGTA, for 1 h, followed by washing and interaction with *R. prolixus* explanted guts for 15 min. The interaction rate was reduced in the presence of 0.5 µM 1,10-phenanthroline (79 ± 5.95%), 1 µM EDTA (74 ± 12.7%) and 1 µM EGTA (68 ± 15.65%) in comparison to control parasites. 1,10-phenanthroline presented a dose-dependent reduction on the adhesion. In addition, anti-gp63 antibodies (1:500) reduced the adhesion to the insect posterior midgut in (65% ± 14.57%) less than control. In relation to the characterization of active peptidases in *R. prolixus*, 10 stomachs or intestines from unfed adult insects after the last ecdysis (~30 days) were explanted and pooled in buffer 50 mM Tris–HCl (pH 6.8). The proteolytic profile revealed bands with peptidase activity only in the intestine of adult males and females that were identified after 24 h of incubation at acid pH (4.5), and were fully inhibited by E-64. Moreover, enzymatic assays with the substrate Z-Phe-Arg-AMC showed the highest level of activity at pH (4.5). Collectively, our results suggest a possible involvement of gp63 in the interaction between *T. cruzi* and *R. prolixus* midgut. **Supported by:**MCT/CNPq, FAPERJ, CAPES and FIOCRUZ

BQ010 - **TCP5CDH AS A KEY COMPONENT IN BIOENERGETIC PROCESSES AND PARASITE´S INFECTIVITY OF *TRYPANOSOMA CRUZI***

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L-proline fulfills energy requirements of *Trypanosoma cruzi*. Δ^1-pyrroline-5-carboxylate dehydrogenase (TcP5CDH) catalyzes the irreversible oxidation of glutamate gamma-semi aldehyde (an intermediate of proline – glutamate oxidation). TcP5CDH was expressed in heterologous system using a yeast mutant Δ*PUT2,* and in bacteria as TcP5CDH-6xHis fusion protein. Kinetic data were determined both in the recombinant form and mitochondrial lysates (ML) with a highest value for k_{cat}/K_m = 2,3 x 10^6 ± 0,26 M^{-1} s^{-1} using saturated P5C and variable NAD^+ concentrations in ML. TcP5CDH undergoes a hexameric arrangement with values of R_g = 67,19 ± 0,15 Å and D_{max} = 200 Å, as estimated by SAXS measurements and Guinier analysis. This quaternary distribution was confirmed by native gel electrophoresis followed by second dimensional and mass spectrometry analysis. TcP5CDH interacts with mitochondrial inner membrane of *Trypanosoma cruzi* probably through a a-helix structure. The catalytic process is coupled to $NAD(P)^+$ reduction. NADH may feed respiratory chain leading to ATP synthesis through oxidative phosphorylation. Expression of TcP5CDH was up regulated in infective stages of *T. cruzi*. When TcP5CDH was over-expressed in *T. cruzi* cells, oxygen rates and ATP levels were stimulated. By contrast, TcP5CDH mutants showed atypical cellular elongation and increased levels of TcGP82; a surface protein involved in adhesion and invasion processes. Aiming to interfere with parasite's viability through P5C metabolism, a reported inhibitor for aldehyde dehydrogenases, Disulfiram®(DS), was tested. DS exhibited an IC_{50} of 402 nM and 647 nM in proliferative curves of epimastigotes and epimastigotes overexpressing TcP5CDH, respectively thus suggesting a role for this enzyme in DSF effect. Moreover, DS decreased activity of TcP5CDH, as seen by enzymatic test. Overall, our data suggest this enzymatic step as crucial in a protozoan parasite postulating it as a target in further drug screening tests. **Supported by:**FAPESP

BQ011 - **"CHARACTERIZATION OF METHYLGLUTACONYL COENZYME A HYDRATASE, AN ENZYME INVOLVED IN THE LEUCINE CATABOLISM OF *TRYPANOSOMA BRUCEI"***

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Trypanosoma brucei fulfills a complex life cycle through the mammalian host and the insect fly. At this journey, metabolic balance is correspondingly affected when moving from these different hosts. Amino acids represent the main source of energy in procyclic form, which proliferates in the insect vector, while in the bloodstream the parasite utilizes anaerobically glucose. For instance, oxidative phosphorylation is highly active in procyclic form, but is absent from bloodstream cells. To obtain energy from amino acids cells require several catabolic pathways that generate Krebs cycle substrates. Most of these enzymes are poorly characterized in trypanosomes. Here we studied methylglutaconyl coenzyme A hydratase (MGCoA), which generates acetoacetate and acetyl-CoA the Krebs cycle in the leucine catabolism. We detected the expression of MGCoA by using specific antibodies in both procyclic and bloodstream forms. In procyclics, the activity is maintained in normal growing cells. However, the specific-activity increased in stationary cells submitted to hypotonic conditions without large changes in protein levels, suggests a post-translational regulation. This indicate that leucine catabolism is regulated in *T. brucei.* Further experiments are being performed to elucidate which conditions affect MGCoA activity and consequently the leucine degradation. **Supported by:**Fapesp e Cnpq

BQ012 - **INHIBITION OF GLYCOLITIC ENZYMES IN *LEISHMANIA AMAZONENSIS* BY 3-BROMOPYRUVATE**

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Leishmania amazonensis, is a unicellular protozoan parasite, responsible for cutaneous and mucocutaneous lesions in humans worldwide. This microrganism has a digenic life cycle residing as flagellated extracellular promastigotes in the gut of insect vector and as nonflagellated amastigotes in mammalian host macrophages. Various new compounds have been developed as antileishmanial drugs. The pentavalent antimonial sodium stibogluconate is the first line of drugs for leishmaniasis. This compound has toxic side effects and the emergence of resistant strains limit their effectiveness. The second line of drugs, amphotericin B and pentamidine, are used clinically, which have some limited efficacy and are very toxic. For these reasons, improved drug therapy for *Leishmania* spp. infections is still desirable, and the need for newer molecular targets and intervention strategies clear and justified. Therefore, enzymes associated in energy metabolism are important candidates to rational designing of leishmanial therapeutic drugs. Among the glycolytic inhibitors currently under development for potential use in cancer treatment, 3-bromopyruvate (3-BrPA) exhibits promising anticancer activity both in vitro and in vivo. 3-BrPA is an analogue of pyruvate/lactate able to react with thiol (-SH) and hydroxyl (-OH) group of several enzymes, interfering with the energy-yielding pathways. In this work, we evaluated the 3-BrPA mechanism over proliferation and glycolytic enzymes of *L. amazonensis*. 3-BrPA treatment resulted in a reduction in the parasites number, where the IC_{50} 100±16 µM in 96 hours of culture. Microscopic examination of the cultured parasites with 3-BrPA shows parasites shorter than control cells (mainly in 300 µM 3-BrPA) and increased cellular volume was noted among parasites treated at 300 µM, though there is no change in motility. The activities of the glycolytic enzymes, hexokinase (HK) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were reduced in parasites grown for 96 hours in the presence of 100 and 300 µM of 3-BrPA. Western blotting analysis detected the expression of a band of approximately 50 kDa in extracts of these parasites incubated with polyclonal rabbit anti-PFK1. This expression was reduced to 50% (100 µM) and 70% (300 µM) with increasing concentrations of 3-BrPA. Also, treatment of *L. amazonensis* with 3-BrPA 300 µM decreased intracellular ATP in 32%. These data suggest that the drug induces the activation of compensatory mechanisms for metabolic energy production and survival. However, more studies are being conducted to better understand the biochemical and molecular mechanisms involved in the phenomenon. **Supported by:**CAPES, FAPERJ, CNPq

BQ013 - **RELATIONSHIP BETWEEN ECTO-ATPASE ACTIVITY AND INFECTIVITY OF RESISTANT *LEISHMANIA***

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Ecto-ATPases are enzymes located on the cell plasma membrane with their catalytic site facing the external medium. These enzymes are able to hydrolyze tri- or di-phosphorylated nucleoside. Some functions are assigned to these proteins: the acquisition of purine, protection against cytotoxicity of high ATP concentrations, participation in the adhesion and infectivity process, involvement in response to heat shock and multidrug resistance. Promastigote forms of *Leishmania amazonensis* were selected by gradual increase in vinblastine (VBT) concentration and maintained under continuous drug pressure. VBT-resistant *L. amazonensis* proliferated similarly to control parasites. However, they showed abnormalities in the cell form and flagella number. Another characteristic (observed by flow cytometry) was the decreased rhodamine 123 accumulation in the resistant cells, proving the MDR phenotype. We also compared the ecto enzymatic activities in control and resistant *parasites*. The Mg^{2+} dependent ecto-ATPase activity was obtained from the quantitation of ^{32}Pi resulting from hydrolysis of the substrate γ^{32}PiATP. The activities of other ecto-enzymes were given by colorimetric quantification of the complex formed by Pi. The control and resistant *Leishmania* ecto-ATPase activities were 16.5 ±1.5 nmolPi × h^{-1} × 10^{-7}cells and 40.0 ± 4.4 nmolPi × h^{-1} × 10^{-7}cells respectively. However, the other ecto-enzymatic activities did not present this increase. The modulation of this activity was also related to the degree of resistance of the cell. Cells resistant to 10µM and 60µM of VBT have ecto-ATPase activity of 22.8 ± 0.5 nmolPi × h^{-1} × 10^{-7}cells and 33.9 ± 1.3 nmolPi × h^{-} × 10^{-7}cells respectively. *In vivo* experiments showed that both lesion size and parasite burden in mice's ears infected with resistant cells are greater compared to the results obtained by *L. amazonensis* control. **Supported by:**Cnpq, Faperj

BQ014 - ***NEW PERSPECTIVES ON THE ROLE OF IMIDAZOLONEPROPIONASE FROM TRYPANOSOMA CRUZI***

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Amino acids are essential during *Trypanosoma cruzi* life cycle, since they can be used as carbon and energy sources and still can participate in several biological processes that help the parasite to adapt to different changes of environments. Histidine is one of the major amino acids in the excreted fluids and hemolymph of the vector insect. Studies performed in other organisms show that His has important biological functions, such as anti-inflammatory and antioxidant. Despite these, little is known about His metabolism in *T. cruzi*. It was shown by our group that *T. cruzi* is able to incorporate and metabolize His up to urocanate and that for 4-imidazolone-5-propionate (IPA) by urocanase (*Tc*UH). IPA can be converted into α-ketoglutarate in a fast non-enzymatic decomposition reaction, or act as the substrate of imidazolonepropionase (*Tc*IP, EC: 3.5.2.7), which could led to the production of glutamate. In this work, we expressed and used the recombinant protein to obtain antibodies and we describe the subcellular localization and expression along the parasite life cycle of *Tc*IP, which converts IPA in formiminoglutamate. As its substrate is extremely unstable in physiological conditions, we also used the recombinant protein to demonstrate that *Tc*IP is able to form a macromolecular complex with *Tc*UH, able to regulate if histidine will contribute to the Krebs cycle or to the biosynthesis of other amino acids. We found, by western blotting, using anti-IP serum, that the protein is mainly expressed in epimastigotes forms, followed by the tripomastigotes. Moreover, an analysis of the subcellular localization by indirect immunofluorescence, showed us, in all parasite stages, a dotted-citoplasmatic profile. Presently we are comparing the *Tc*IP expression patter together with *Tc*UH, to infer in which situations both enzymes are being expressed concomitantly and in which situations not. This will contribute to unveil the role of histidine and its metabolism in *T. cruzi* biology. **Supported by:**CAPES

BQ015 - **METABOLIC RELEVANCE OF GLUTAMINE SYNTHETASE IN *TRYPANOSOMA CRUZI*: *BIOCHEMICAL AND FUNCTIONAL INSIGHTS***

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Glutamine synthetase (GS) is an ubiquitous enzyme involved in complex cellular functions, such as nitrogen detoxification and glutamine biosynthesis. Glutamine is a key molecule for the production of the cabamoyl-phosphate, glucosamine-6-phosphate and for providing nitrogen to several molecules in many cellular events, such as the purines, pyrimidines and amino acids. In *Trypanosoma cruzi*, glutamate is a substrate of the reaction catalysed by GS. This amino acid can be transported from the extracellular medium and participates in the energy production, oxidative stress resistance, metacyclogenesis, and osmoregulation. Therefore the GS and other enzymes involved in the degradation of this amino acid could be important for the parasite viability. The recombinant GS from *T. cruzi*, was expressed in *E. coli* and purified in active form. The kinetic parameters V_{max}, K_m, K_{cat}, E_a, the cofactor influence, specificity for substrates and the optimum pH were established by using two different methods. The existence of a quaternary structure was revealed by non-denaturing electrophoresis and size-exclusion chromatography, constituting the active complex of a 320 kDa octamer. Surprinsingly, the *Tc*GS subcellular localization, in all parasite stages, was citoplasmatic and nuclear/kinetoplastid. Amastigotes presented the higher transcript amount, protein and enzyme activity, leading us to infer a role in this stage. The treatment of infected cells with a *Tc*GS inhibitor, L-Methionine sulfoximine, reduced the trypomastigote bursting in CHO-K_1 infections (IC_{50} = 20.02 µM, non-toxic up to 10 mM in CHO-K_1). In addition the amastigote stage has the higher transcript amount, enzyme activity, as well as the higher *Tc*GS expression. As intracellular amastigotes are dependent on amino acid degradation as energy sources, producing NH_4^+, and GS inhibition diminished the infection yields, we propose that *Tc*GS is relevant in the intracellular cycle, due to its role on ammonium detoxification. **Supported by:**CNPQ, FAPESP, CAPES

BQ016 - ***LEISHMANIA AMAZONENSIS* LIPID MICRODOMAINS: LDL ENDOCYTOSIS AND PHLEBOTOMINE GUT ADHESION**

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DRM (detergent-resistant membranes) or lipid rafts are lipid-enriched microdomains in which cholesterol is the principal component. Recent studies showed that it can be isolated from different cell types and are involved in cholesterol transport, signal transduction and endocytosis, including LDL uptake. The aim of this work is to study the role of *L. amazonensis* DRM in LDL uptake and parasite adhesion on its vector, *Lutzomyia longipalpis* gut. To study LDL receptor-mediated endocytosis and to confirm the presence of LDL-receptor, we isolate parasites membranes and performed the specific binding assay. LDL-I^{125} bound to the membranes in a specific manner and its decrease in excess of LDL. The membranes were also subjected to 7% SDS-PAGE followed by ligand-blotting using LDL, anti-apolipoprotein B and calf hepatocyte membranes as positive control. LDL was bound to a protein with a molecular weight around ~60 kDa, different from the receptor found in mammals. This protein was also found in flagellar pocket using LDL-Au 50nm in transmission electron microscopy. To investigate the DRM-mediated endocytosis of LDL, parasites were treated with various concentrations of MBCD, a lipid rafts disruptor, and then incubated with LDL-I^{125}. Treated cells show a significantly reduced LDL uptake in a dose-dependent manner. To investigate the presence of LDL-receptor in DRM, 1 x 10^9 cells were lysed, incubated at 4°C in Triton X-100 1% and subjected to sucrose density gradient. The gradient fractions were subjected to a dot-blot and we identified Flotillin, a DRM marker, content in the 4-7 fractions and LDL-receptor in 5-9 fractions. In order to verify the role of DRM in plhebotomine interaction, we pre-treated 1 x 10^7 cells with 20mM of MBCD and incubated with *L. longipalpis* gut. After one hour, we observed a reduction of 69% in the number of parasites attached at the gut. These results could provide better tools for understand the interactions between parasites and their hosts. **Supported by:**CNPq, FAPERJ, IFS

BQ017 - **EFFECT OF PLATELET-ACTIVATING FACTOR (PAF) AND CELL SIGNALING MODULATORS ON THE INTERACTION OF LEISHMANIA CHAGASI WITH A CELL LINE DERIVED FROM LUTZOMYIA LONGIPALPIS**

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Protozoan parasites of the genus *Leishmania* are responsible for several forms of leishmaniasis. *Leishmania* spp. are transmitted by the bite of phlebotomine sandflies. *Lutzomyia longipalpis* is the most important vector of American visceral leishmaniasis, as it is the vector of *Leishmania chagasi* in the New World. Studies on the interaction of the parasite with its vector are extremely important for the knowledge of parasite biology, and may contribute for the development of new drugs for treatment of the disease. Cell lines are suitable models for studying this interaction, because they are easier to manipulate and to maintain in laboratory than the insects themselves. Platelet-activating factor (PAF) is a phospholipid with diverse physiological and pathophysiological actions, including interaction between *Leishmania amazonensis* and mouse peritoneal macrophages *in vitro*. Here we describe the effects of PAF, as well as a couple of cell signaling modulators, on the interaction of *L. chagasi* with a *Lu. longipalpis* cell line (LL5) *in vitro*. *L. chagasi* promastigotes were grown in a complex medium supplemented with 10% heat inactivated fetal bovine serum (HI-FBS). Insect cell line LL-5 was 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 adherent insect cells in MM-FBS at a 10:1 ratio. The parasites and/or the insect cells were maintained for 1 h in the absence or in the presence of PAF and/or inhibitors for PKC (BIS) or CK2 (TBB), before the interaction assays. After 2-h interaction, the insect cells were washed with PBS, fixed with methanol and stained with Giemsa. When the parasites were pre-treated with PAF, there was an increase in the association indices and when LL-5 insect cells were pre-treated with PAF, there was a decrease in the association indices, as compared to the control systems. The pre-treatment with PAF antagonist (WEB 2086) or BIS completely abolished the effects promoted by PAF. **Supported by:**FAPERJ, CNPq, CAPES, INCT-EM

BQ018 - **ACYL-COA: CHOLESTEROL ACYLTRANSFERASE IN *LEISHMANIA AMAZONENSIS* AND *LEISHMANIA INFANTUM***

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In order to store cholesterol (CHO) without suffering any damage, cells have many biological mechanisms. One is the CHO esterification, catalyzed by Acil-CoA: cholesterol acyltransferase (ACAT) that converts CHO into cholesteryl ester (CHOE) by introducing a fatty acid chain to CHO. Trypanosomatids including those from the *Leishmania* genus, have incomplete *de novo* lipid synthesis. Therefore, these organisms avidly take up lipids including cholesterol, from the external environment. It was demonstrated by our group that *L. amazonensis* utilizes human LDL as a CHO source which is accumulated in a dose-dependent manner. The aim of present study is to verify the presence and the activity properties of the ACAT in *L. amazonensis* and *L. infantum*. ACAT enzyme activity in promastigotes and amastigotes forms of *L.amazonensis* and promastigotes forms of *L. infantum* was determined by incubation of parasites at 4 °C and 28 °C in presence of LDL for 30 min and then with ^{3}H-Free Fatty Acid associated with bovine serum albumin. Lipids were extracted at different times and characterized by HPTLC and the spots corresponding to esterified CHO were elluted from silica and CHOE-associated radioactivity was measured by scintillation counting. We verified that the ACAT activity occurs in a time-dependent manner at 28°C and it is severely inhibited at 4°C. Furthermore, experiments were also performed to characterize the best temperature and pH of the enzyme in intact cells and cell homogenates by making the reaction at different conditions. The results shows a best activity at pH 7,5 and a temperature around 32°C. To confirm the presence of ACAT, 1 x 10^{7} cells are fixed and incubated with anti-ACAT for 1h at 28ºC. Images are acquired using epifluorescence microscope. The ACAT green fluorescence can be observed along the parasite body indicating that enzyme is distributed in the cell. These results could provide better tools for understand the interactions between parasites and their hosts. **Supported by:**PIBIC/UFRJ, CNPq, FAPERJ, IFS

BQ019 - **ACTION OF INHIBITORS OF NUCLEOSIDE HYDROLASE IN *L. CHAGASI***

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The *L. donovani* nucleoside hydrolase was identified as a nonspecific and have specificity for a wide range of substrates, inosine, guanosine, adenosine, uridine and cytidine with a slight preference for adenosine and inosine. Guanosine is not a good substrate. The catalytic specificities differ substantially from *L.major*, relative values of K_{cat} for the *L. donovani* enzyme with inosine, adenosine, guanosine, uridine and cytidine as substrate. In the present study we have analyzed a number of potential nucleoside hydrolase inhibitors, since if this enzyme is inactivated the parasite is not able to multiply and thus will not be able to maintaining infection.
Preliminary results showed that two compounds had low Ki and IC 50%, when we added the inhibitor only at 0h, whereas another compound has an IC 50% in the nanomolar range, but a Ki of greater than 5uM in kinetic assays. So, we decided to monitor the parasite growth *in vitro* during four days, using for two compound concentrations ranging from 0,05 µM to 250 µM and additionally, from 0,05 nM to 250 nM for a third compound, in this assay the addition of all inhibitors was done at 0, 24, 48 e 72 hours. All the compounds inhibited the parasite growth in a dose-dependent manner.
Our results may be useful in the design of anti-leishmania agents, since the existing drugs to treat leishmaniasis were all developed for other indications and the treatment is very expensive, painful, uses the intravenous pathway and has severe toxic inside effects that can be lethal.
Supported by:CNPQ

BQ020 - **Δ^1-PYRROLINE-5-CARBOXYLATE SYNTHETASE, IS ONE KEY ENZYME INVOLVED IN PROLINE BIOSYNTHESIS IN *TRYPANOSOMA CRUZI***

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Besides the fact that proline is a major carbon source in most trypanosomatids, several others roles have been postulated for this amino acid in *Trypanosoma cruzi*. Proline participates in osmoregulation, metacyclogenesis, cell invasion, differentiation of epimastigotes to trypomastigotes and resistance to different types of stress. Proline levels are affected by *de novo* synthesis and degradation. In the present work, we are focusing on proline biosynthesis and hypothesize that it is solely synthesized from glutamate which is phosphorylated to gamma-glutamyl phosphate and afterwards reduced to glutamate semialdehyde by a bifunctional enzyme Δ1-pyrroline-5-carboxylate synthetase (P5CS) (EC: 1.2.1.41 & 2.7.2.11). We cloned and sequenced the gene coding the *Tc*P5CS. The conditions were found to successfully express this enzyme in *Escherichia coli* BL21 Codon Plus (pGro7) strain, by using pET28a(+) vector. The expression was induced by adding 0,5mM of IPTG. The addition of substrate (100 uM glutamate) resulted in an improved expression. The optimal temperature resulted to be 37°C, conditions in which the maximum expression of the soluble enzyme was reached within 4 hours incubation. The recombinant protein in its soluble form was purified by affinity chromatography using NTA-Ni^{2+} resin. Its apparent molecular mass was determined by SDS-PAGE as being of 81 kDa as expected for *Tc*P5CS. Kinetic analysis of parasite extracts and the recombinant proteins are being performed. Additionally the recombinant protein is being used to produce anti-TcP5CS polyclonal monospecific antibodies in order to determine its subcellular location and expression levels of *Tc*P5CS in all stages of the life cycle of *T. cruzi* by western blot analyses. **Supported by:**CNPq

BQ021 - **SIGNALS MEDIATORS OF ARGININE TRANSPORTER EXPRESSION IN *LEISHMANIA (L.) AMAZONENSIS***

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During it life cycle *Leishmania* is exposed to different environments such as the sandfly vector midgut (25°C – pH 7.0) and the macrophage parasitophorous vacuole (pH 5.0 - 34°C). Arginine is a common substrate to arginase and nitric oxide synthase. These enzymes are present in both host mammalian cell and parasite and their activities may drive the success or the control of macrophage infection. Previously we showed that arginine uptake in *Leishmania (L.) amazonensis* is made by an amino acid porter 3-like protein (AAP3), coded by two gene copies arranged *in tandem* in the genome. The transcripts, AAP3 5.1 and AAP3 4.7, present 98% of ORF identity, but differ in the 5' and 3' UTR's. We also verified that AAP3 5.1 transcript is more abundant than AAP3 4.7 and is regulated through promastigote growth curve and during arginine starvation (Castilho-Martins et al, 2011). Here, we evaluated the existence of other signals during metacyclogenesis or differentiation to amastigote that could also regulate the expression of AAP3. For this, we submitted log-phase promastigotes of *L.(L.) amazonensis* to 4 hours arginine starvation in EBSS medium, at 25°C or 34°C, in combination to pH 5.0 or pH 7.0. Also, parasites at the same conditions were maintained in the presence of arginine (40 μM). Thereafter, total RNA extraction was carried out with Trizol® and the two AAP3 transcripts were quantified by RTPCR, normalized by GAPDH mRNA. We confirmed that the AAP3 5.1 is more abundant than the AAP3 4.7 in starvation condition and besides, the amount of the AAP3 5.1 transcript increased during L-arginine starvation at 34°C in both pHs compared to 25°C. The temperature increase also led to a raise of AAP3 4.7 expression. Our results indicated that the change of temperature and pH, besides starvation of arginine, might be signals to a differential expression of AAP3 mRNA, mainly the AAP3 5.1 copy and this could be a strategy used by the parasite for infection success. **Supported by:**FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO - FAPESP

BQ022 - **NOVEL TOOLS TO IDENTIFY PLASMODIAL PROTEINS ON THE SURFACE OF INFECTED ERYTHROCYTES.**

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Plasmodium falciparum, the causative agent of human malaria, is able to export variant proteins to the surface of its host cell. These proteins have different tasks in terms of being responsible for cell adhesion, nutrient transportation or so far unknown function. Further the malaria parasite possesses at least two routes for trafficking proteins to the surface of the erythrocyte – a PEXEL motif dependent and independent mode. Consequently it is hard to predict, which and how many proteins are sorted to the surface. In this sense we applied the SELEX technique (Systematic Evolution of Ligands by Exponential Enrichment) to identify novel secreted proteins. SELEX uses nucleic acid ligands with high-affinity and specificity, denominated as aptamers, which are processed by high-flux *in vitro* screen of iterative cycles. For this, we used a random oligonucleotide library of 10^{13} different DNA molecules. This library was used as template in a conventional PCR reaction with sense and anti-sense oligonucleotides labeled with triple biotin and FITC, respectively. Subsequently, single-stranded DNA (FITC-labeled) was separated through a denaturing gel and further used in an *in vitro* assay against infected synchronized erythrocytes. The interaction between DNA-ligands and parasite specific proteins was visualized by live cell fluorescence microscope. Subsequently, the deriving aptamers were cloned and the consensus sequence determined. In the following these aptamers will be used to selectively pull down parasite derived secreted proteins and proliferation assays to analyze the viability of the deadly human pathogen. **Supported by:**FAPESP

BQ023 - **NUCLEAR FRACTION PREPARATION OF TRYPANOSOMA CRUZI EPIMASTIGOTES FOR SUBPROTEOME ANALYSIS.**

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T. cruzi nuclear machinery for cell division has features that are not shared by other eukaryotes. For instance, throughout *T. cruzi* cell division the nuclear membrane is preserved and the chromosomes do not condense. The objective of this work is to establish a protocol for purification of nucleus from epimastigote form of *T. cruzi* to proteomic purposes. For that, epimastigotes of the CL Brener strain were cultured in LIT medium, harvested and afterwards 10^{11} parasites were lysed in hypotonic solution with 2% NP40 and Dounce homogenization. The lysate was deposited on a sucrose 1 M layer and centrifuged 2,000 *g* at 4^{o}C for 10 min, then the pellet was ressuspended in sucrose 1.9 M and subjected to ultracentrifugation on a discontinued gradient using three layers of sucrose solution (2.01 M, 2.10 M and 2.3 M, respectively). The sample was centrifuged for 3 hours at 141,000 *g* at 4^{o}C originating three main fractions from lower to higher density: membranes and low density structures, kinetoplasts and flagellum, and nuclei. The purity of the nuclear fraction was verified by fluorescence microscopy where the DNA was stained with DAPI and compared with bright field microscopy. This analysis showed that the nuclear fraction contained a high amount of nuclei with absence of contaminants, indicating that the ultracentrifugation protocol was successful. For protein extraction, the purified nuclei were lysed in a solution composed of 7 M urea, 9 M thiourea and analyzed using SDS-PAGE to assess protein integrity. We observed differences in the protein profile between the nuclear extract and extracts of the other fractions obtained from sucrose gradient as well as differences from epimastigote total extract. Wide range pH (3-10) 2-DE gels were used to produce protein maps from nuclear fraction as well as total *T. cruzi* extract. We are presently performing identification of protein spots from the 2-DE gels. In addition the nuclear proteins will be subjected to LC-MS/MS analysis.â€‹â€‹ **Supported by:**CNPq, CAPES, FAPEG

BQ024 - ***TRYPANOSOMA CRUZI* TRYPAREDOXIN II INTRACELLULAR LOCALIZATION DURING THE CELL CYCLE**

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New chemotherapies targeting *Trypanosoma cruzi* are urgently required. One potential target is the parasite specific system employed to detoxify reactive oxygen species. For peroxide metabolism, a series of enzymatic pathways dependent on the trypanosome thiol trypanothione have been reported. The cytosolic pathway tryparedoxin-dependent (TcTPNI) has been characterized. Another tryparedoxin, TcTPNII, with homology to TcTPNI has been identified from the parasite genome database. The truncated recombinant TcTPNII (without its 22 C-terminal transmembrane amino acids) shows tryparedoxin activity being able to transfer reducing equivalents from trypanothione to peroxiredoxins. This protein appears to be located to the glycosomal, endoplasmic reticulum or to the outer mitochondrial membrane. The aim of these experiments was to determine TcTPNII intracellular localization and if this changes along the cell cycle. In order to do so, epimastigotes (Y strain) were transfected with pTEX-5'TcTPNII-RED-3'TcTPNII vector – containing both domains surrounding the RFP. After drug selection, cells were incubated in LIT medium and at 0, 3, 7, 18 and 30h were collected by centrifugation, washed in PBS and analysed by Confocal microscopy. During this time-range no differences regarding TcTPNII location were observed. Experiments are being conducted to determine its exact intracellular localization. **Supported by:**FAPESP and Capes

BQ025 - **MITOCHONDRIAL TRYPAREDOXIN PEROXIDASE IS LOCATED TO THE MITOCHONDRIAL MEMBRANE OF *TRYPANOSOMA CRUZI***

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Trypanosoma cruzi is the etiologic agent of the Chagas disease, a parasitosis with great clinical importance and until now there is no vaccine and effective treatment. Thus, research aim at the search for new therapeutic targets in order to develop a more specific therapy. Among these targets, the antioxidant system of the parasite has emerged, and key enzymes of this system as cytosolic and mitochondrial tryparedoxin peroxidases (TcCPx and TcMPx, respectively), has increasingly shown its importance in parasite survival. The cytosolic pathway in which TcCPx participates have already been characterized, however, the mitochondrial one not yet. The objective of this study was to determine the exact location of TcMPx and thus be able to suggest to which proteins this enzyme interacts. Therefore, we obtained mitochondrial membrane fraction (MMF) of *T. cruzi* Y strain, and from this fraction, experiments of oxygen consumption, citrate synthase activity and detection of the expression of this protein were performed. TMPD / Ascorbate supported oxygen consumption, indicating that MMF successfully obtained. In order to prove that MMF was free from mitochondrial matrix enzymes, citrate synthase activity was performed. This activity was observed in intact cells but not in MMF. Thereafter, western blotting was performed for detection of TcMPx expression in MMF, and the result showed the presence of this protein not only in the whole-cell and supernatant of cellular lysis proteic extracts, but also in the MMF. The detection of this enzyme in the mitochondrial membrane opened new perspectives in relation to TcMPx interatome, and experiments are being carried out to finalize the characterization of the mitochondrial antioxidant system of *T. cruzi*. **Supported by:**FAPESP and Capes

BQ026 - **MITOCHONDRIAL O2 CONSUMPTION RATES AND H2O2 RELEASE ARE HIGHER THAN IN CONTROL CELLS DURING DNA REPAIR**

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Trypanosoma cruzi epimastigotes have to deal with the reactive oxygen species (ROS) in the invertebrate host intestinal tract derived from the degradation of hemoglobin that generates high levels of heme. The excess of ROS could have deleterious effects to cells since they can oxidize several molecules such as lipids, carbohydrates, proteins and nucleic acids. Like most living organisms, *T. cruzi* is susceptible to oxidative stress; hence DNA repair is essential for its survival and establishment of the infection. *T. cruzi* has only one mitochondrion and nowadays, it is clear that it has an important role besides the production of ATP. Herein, the extension to which the mitochondrion contributes to parasite survival during DNA repair was evaluated. Cells (CL Brener strain) were treated with lethal H_2O_2 concentrations (200mM) for 15 min. Afterwards cells were incubated for different periods of time (0-24h) in old LIT medium to allow DNA repair. During this time-range O_2 consumption rates (Hansatech Oxygraph) and H_2O_2 release (Amplex red® technique) were determined. Overall, treated cells had higher respiratory rates and lower respiratory control than control cells. Regarding H_2O_2 release, an increase of ~80% is initially observed followed by a gradual decrease that reached values comparable to the control upon 24h of incubation. It can be suggested that in cells submitted to an oxidative stress condition, during DNA repair, mitochondria function is slightly different from control cells probably due to the urgent need of ATP production. Experiments are being conducted to prove this hypothesis. **Supported by:**FAPESP and Capes

BQ027 - **SCHIFF BASE STRUCTURALLY RELATED TO NIFURTIMOX AND BENZNIDAZOLE INHIBITS O2 CONSUMPTION AND INCREASES H2O2 RELEASE IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES**

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Chagas disease (CD) treatment leads to a series of side toxic effects, besides the lack of effect on the chronic phase. The biochemical and genetic diversity among *Trypanosoma cruzi* strains contributes to the differences in resistance/sensitivity to the drugs used in treatment. The search for a more specific treatment and consequently less toxic to the vertebrate host is a priority. Changes in the structure of nifurtimox and benznidazol, commonly used in CD treatment, can lead to an enhancement of the therapeutic efficacy. Herein, the trypanosomicidal effect of one of these derivatives was evaluated. O_2 consumption rates determined in a Hansantech Oxygraph and H_2O_2 release using Amplex red® was assayed in *T. cruzi* epimastigotes (Y, Tulahuen 2 and CL Brener strains). The IC_{50} for the Schiff base was determined and cells were incubated in the presence of sub-lethal concentrations. At the *log* phase cells were collected by centrifugation, washed and resuspended in PBS and the number of cells determined in a Newbauer chamber. The Y strain had the lowest IC_{50} for this Schiff base (3.78 ± 0.03 mg/ml) when compared to Tulahuen 2 and CL Brener strains (8.75 ± 0.07 and 9.45 ± 0.07 mg/ml, respectively). Preliminary results show that an inhibition of succinate-supported O_2 occurs (~80%) in Y and Tulahuen 2 strains. Regarding H_2O_2 release, in CL Brener there was an increase in ~400% in treated cells compared to control. Experiments regarding mitochondrial bioenergetics are being performed, but so far the results suggest that the cytotoxic effect of this Schiff base is ROS-mediated. **Supported by:**FAPESP, CNPq and FAPEMIG

BQ028 - **INVOLVEMENT OF GP63 FROM *LEISHMANIA BRAZILIENSIS* AND *LEISHMANIA INFANTUM* IN THE INTERACTION WITH THE INVERTEBRATE HOST**

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The highly abundant surface metallopeptidase of *Leishmania*, gp63, contributes to a myriad of well-established functions for *Leishmania* in the interaction with the mammalian host. However, despite this molecule being abundantly expressed on the surface of promastigote forms, found in the insect vector, little is known about the functions performed by this molecule in the phlebotomine sandfly. Molecular analysis revealed conflicting and inconclusive data about the possible role of this glycoprotein in the interaction of *Leishmania* with the invertebrate host. Our research group, using biochemical approaches, has demonstrated that gp63 molecules from several trypanosomatids are implicated in the adhesion to the insect epithelial cells. Here, we analyzed the role of gp63 in the interaction of *L. braziliensis* and *L. infantum* with their respective insect hosts, *L. intermedia* and *L. longipalpis*. The dissected insect guts, opened longitudinally, were pretreated with PG and placed to bind with the parasites. In parallel, promastigotes of *L. infantum* were pretreated with anti-gp63 antibodies or with metallopeptidase inhibitors, while promastigotes of *L. braziliensis* were subjected only to the anti-gp63 treatment. Thereafter, the parasites were placed to interact with dissected insect guts. As expected, PG virtually abolished *Leishmania* ability to bind to the insect guts. Interestingly, we observed that all treatments related to gp63 also provoked a pronounced decrease in this binding assay. Moreover, the culture supernatant of *L. braziliensis* was concentrated by precipitation with ammonium sulfate and analyzed by SDS-PAGE. We observed a proteolytic degradation around 63 kDa, which corresponds to the gp63 enzyme already identified and characterized in several *Leishmania* species. The determination of the functions and characterization of gp63 of these parasites can contribute to the understanding of the physiological role played by this peptidase in *Leishmania.* **Supported by:**MCT/CNPq, FAPERJ, CAPES and FIOCRUZ