A COMPARISON OF THE IN VIVO AND IN VITRO ACTIVITY OF PENTOSTAM OF CHINESE ORIGIN AND GLUCANTIME AGAINST LEISHMANIA BRASILIENSIS ISOLATED FROM PATIENTS WITH CUTANEOUS LEISHMANIASIS IN VITÓRIA, ES, BRAZIL

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Pentavalent antimonials have been the standard agents in the treatment of cutaneous leishmaniasis in Brazil since the late forties. To date, there is no clear assessment on the emergence of resistance to pentavalent antimonials in Leishmania and the role of those parasites in treatment failures. Until recently, glucantime was the most widely used first line anti-leishmanial drug in Brazil when a sodium stibogluconate of Chinese origin, another pentavalent antimonial, was put on the market. The purpose of this study was to assess the involvement of resistant Leishmania parasites in the failure to cure leishmaniasis patients. Two groups of patients with cutaneous leishmaniasis were administered standard treatments with either one or the other drug. The two groups were then compared in relation to the cure rate of patients and the corresponding in vitro sensitivity of the parasite to the two drugs. Overall, the cure rate was similar for patients treated with either glucantime or sodium stibogluconate. Out of a total of 27 patients, two relapsed after being treated with sodium stibogluconate and four relapsed after a treatment with glucantime. To assess the role of the intrinsic sensitivity of the parasite to the two drugs, kill curves in vitro (IC50) were performed on those Leishmania braziliensis showing differences in vivo, patients who relapsed versus patients who cured (the control group). Present data shows low IC50 values for all control parasites tested: 4 with glucantime and 3 with sodium stibogluconate. Two out of three parasites from patients who relapsed after a treatment with glucantime showed elevated IC50 values as did the one parasite evaluated from a patient resistant to a sodium stibogluconate treatment. More parasites are being tested in order to establish if this difference is statistically significant.

A COMPUTATIONAL STUDY (SAR) OF THE MESOIONIC AGENTS 1,3,4-THIADIAZOLIUM-2-AMINIDE CLASS IN LEISHMANIA AMAZONENSIS PROMASTIGOTES AND AXENIC AMASTIGOTES

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The in vitro antileishmanial activity of a series of fourteen 1,3,4-thiazolium-2-aminide mesoionic class was evaluated against Leishmania amazonensis promastigote. Six of these derivatives had been tested in this study in the axenic amastigote form of the parasite. The preliminary results were very promising showing significant in vitro anti-Leishmania activity of the derivatives tested, some of them with comparable or highest activity value than the pentamidine in both, promastigotes and amastigotes forms of L. amazonensis. The agents tested showed a significant difference between the axenic amastigote and promastigote assays. Parallely, the reference antileishmanial agent pentamidine were less active for amastigotes than for promastigotes.

Theoretical calculations based on the semi-empirical AM1 method were performed with all compounds and theoretical electronic parameters like HOMO and LUMO energies, charges and dipole moments for each molecule were obtained. In general, the electronic factors, are responsible for alterations of the physical and chemical properties of the compounds, reflecting their biological activity. However, we could not correlate these electronic factors with the differences in the effectiveness observed among the substituted group. As part of our research project on chemotherapy, we are going to continue screening other 1,3,4-thiazolium-2-aminide derivatives with other substituent as another aproach to correlate chemical structure with activity.

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ACTIVITY OF 7-EPICLUSIANONE AGAINST BLOOD TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI

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7-Epiclusianone, a polyprenylated benzoquinone, isolated in large quantity from the fruits of *Rheedia gardneriana* Miers (Clusiaceae) was evaluated in five biological assays to assess its pharmacological potential. It was active *in vitro* against trypomastigotes of *Trypanosoma cruzi* (Y strain). Ninety-two percent of the parasites were cleared from blood after 24h incubation at 4°C at a concentration of 500mg/mL. However, 7-epiclusianone was not active on *T. cruzi* infection *in vivo* when administered orally in mice either at a high single dose (500 mg/kg/body weight) or in a four days schedule with 100 mg/kg/ body weight/day. This compound displayed an IC$_{50}$ of 25 ppm in the brine shrimp lethality assay, a model that has been used as a surrogate assay for cytotoxic activity. It was inactive against the fungus *Cladosporium sphaerospermum*.

Table 1: *In vitro* activity of 7-epiclusianone, and the control drug gentian violet on trypomastigote forms of *Trypanosoma cruzi* present in blood of experimentally infected mice.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (mg/mL)</th>
<th>Trypanocidal Activity (% ± S.D.) $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-epiclusianone</td>
<td>500</td>
<td>92±1.0</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>38±3.7</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>17±1.2</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without drug</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Gentian violet</td>
<td>7.5</td>
<td>47±1.7</td>
</tr>
<tr>
<td>DMSO</td>
<td>2.5%</td>
<td>2±4.8</td>
</tr>
</tbody>
</table>

$^a$ The experiments were run in duplicate and repeated twice. The activity is expressed as percent reduction of the parasite number in infected murine blood ± standard deviation (S.D.).

Financial support: Pronex, Fiocruz, CNPq, IFS

CH-4

ANTILEISHMANIAL ACTIVITY OF THE SCHIFF-BASE-FORMING COMPOUND TUCARESOL

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In the search for new drugs for the treatment of leishmaniasis there has been a major emphasis on biochemical and molecular targets, for example trypanothione reductase and cysteine proteases, and the identification of inhibitors by rational design or empirical screening. The ability of *Leishmania* parasites to survive and multiply in humans is also dependent upon the adaptation of parasites to establish an infection in macrophages and on host immuno-suppression. These particular elements of the host-parasite interaction indicate that immunostimulation represents another rational approach to the treatment of leishmaniasis. Immunomodulators that have been used in studies on both visceral and cutaneous leishmaniasis, either alone or in combination with chemotherapeutic drugs, include the endogenous biologicals IFN-γ GM-CSF and IL-12, the bacterial and fungal derivatives, muramyl dipeptide, trehalose dimycolate and glucan, and the synthetic compounds, levamisole, the lipoidal amine CP-46,665-1, tuftsin and polyinosinic-polycytidylic acid. The Schiff-base-forming drug tucaresol has been shown to enhance T-helper cell activity and the production of IL-2 and IFN-γ in mice and humans. The compound probably acts by providing a co-stimulatory signal to T cells, supplementing costimulation provided by receptors on antigen presenting cells such as macrophages. The down regulation of specific macrophage receptors is a characteristic effect of infection by *Leishmania*. We have examined the effect of tucaresol in models of infection of *Leishmania donovani*. Tucaresol showed no direct antiparasitic activity against *L. donovani* amastigotes in either peritoneal or bone marrow macrophages *in vitro* at concentrations between 30 and 1nM. However, against *L. donovani* in BALB/c mice at doses of 20 mg/kg x 5 during days 7-11 of infection, a dose level found to be most effective in viral models of infection, a 30% suppression of parasite load in the liver was observed. No activity was observed when mice were dosed with tucaresol during days 1-5 of infection. Subsequent experiments on infected mice treated during days 7-11 of infection showed a bell-shaped dose-response curve with an optimal dose of 5 mg/kg causing 60% suppression and limited activities at the extremes of the dose range (80 and 1.25 mg/kg). This response was not altered by levels of infection. There was additive but not synergistic activity with Pentostam.

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR)
ANTIPARASITIC ACTIVITY AGAINST DIFFERENT STAGES OF *TRYPANOSOMA CRUZI* OF BIOMOLECULES ISOLATED FROM *AZORELLA COMPACTA* (UMBELLIFERAE)


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*Trypanosoma cruzi* is the aethiological agent of Chagas’ disease, an illness of considerable morbidity and mortality in Latin America. Nowadays there is no successful treatment, and only few drugs are active in acute phase and they don’t have activity against intracellular forms of parasite and induced several adverse effects. Search of new drugs against *T. cruzi* is therefore an urgent need. In this study we are evaluating by bioassay-guided protocol the trypanocidal activity of molecules isolated from *Azorella compacta*, Umbelliferae family, an autochthonous medicinal plant of Andes Mountains of Northern of Chile. The trypanocidal activity of diterpenoid type compounds was measured against the epimastigote, trypomastigote and intracellular amastigotes stages of SPA-14, Tulahuen and G strains, CL Brenner and D-11 clone of *Trypanosoma cruzi*.

Two diterpenoides with a carbon skeleton known as mulinano and Yaretane (Loyola et al. 1996) showed an high trypanocidal activity, expressed as percentage of mortality, with lethal dose 50 (DL50) of 0.020 mM for Azorellanol against trypomastigote forms and 0.037 mM of mulin-11-13-dien-20-oico acid against epimastigote forms.

The trypanocidal activity over intracellular forms show that growth was inhibited ~60% in different strains assayed, with a DL50 for HeLa and Vero cells of 0.139 mM and 0.132 mM, respectively. Further studies will done in order to increase a biological activity for this molecules.

Supported by International Foundation of Science (IFS), FONDECYT, Chile.

ANTIPROLIFERATIVE EFFECTS OF AJOENE IN PROMASTIGOTES AND AMASTIGOTES-LIKE FORMS ON SEVERAL *LEISHMANIA* SPECIES

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Ajoene [(E,Z)-4,5,9 Trithiadodeca 1,6,11 Triene 9-Oxide], the main active compound derived from garlic, displays tripanolytic and antimicotic activities. This effect seems to be related to its ability to selectively inhibit phospholipid synthesis in these pathogens. Since the biosynthetic pathways in these organisms are in general very similar, we decided to evaluate the effect of ajoene in several Leishmania species, axenically grown: *L. mexicana*, venezuelensis (Lmv: MHOM/VE/80/H16), *L. mexicana amazonensis* (Lma: M112, IFLA/BR/67/PH8) and *L. donovani chagasi* (Ldch: MHOM/BR/74/PP75). Ajoene showed a potent, dose-related, leishmanicide activity against the promastigotes form of all the above mentioned species. Effective concentrations of ajoene were in the range of 0.3 to 8 µM, with an IC50 of 1 µM. Above 10 µM, ajoene induces 100% lysis after 96 hours of incubation, while 30 µM and 100 µM produces total lysis of the promastigotes and amastigotes forms of *L. amazonensis* in 72 and 24 hours respectively.

Electron microscopy studies show marked alteration of mitochondrial membranes and the formation of large autophagic vacuoles which, as described above, finally leads to parasite death. Since ajoene has been used topically in humans, as antimicotic, the present findings open the possibility of clinical use of the drug to treat localized cutaneous leishmaniasis.

ANTI-TRYPANOSOMATID TARGETS: GLYCOLYSIS AND PROTEIN PRENYLATION


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Bloodstream *T. brucei* and possibly *T. cruzi* amastigotes use glycolysis as their main source of energy. Using the x-ray structures of trypanosomatid glyceraldehyde phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase (PGK), determined in our lab, we are designing adenosine analogs as inhibitors of trypanosomatid GAPDH, and to date our most potent adenosine analog inhibits *L. mexicana* GAPDH within a Kp of 200 nM, nearly a million-fold more potent than our lead compound adenosine. Progress toward the development of PGK inhibitors will also be discussed. The toxicity of these compounds to parasites will be presented.
We have shown that protein prenylation (farnesylation and geranylgeranylation) occurs in trypanosomatids, and we have purified to homogeneity the protein farnesyltransferase from *T. brucei*. Inhibitors of this enzyme block protein farnesylation in trypanosomatids and sub-micromolar concentrations are lethal to cultured *T. brucei*, *T. cruzi*, and *L. mexicana*. Since there is an enormous effort to develop protein farnesyltransferase inhibitors as anticancer agents, one may be able to extend the medicinal chemical and clinical pharmacological studies currently in progress to the development of anti-trypanosomatid agents.

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**CH-8**

**BIOCHEMICAL CHARACTERIZATION OF THE RESISTANCE DEVELOPMENT OF *LEISHMANIA MEXICANA* TO GLIBENCLAMIDE**

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The frequency observed in the lack of response of *Leishmania* sp. to various drugs, including those used for the treatment of the disease, is related to the development of resistance and emphasizes the need for the comprehension of the biochemical and molecular mechanisms involved in this process. In previous studies we had demonstrated a susceptibility (Gs) of *Leishmania* to K⁺ transport blockers as glibenclamide (GLIB) with EC_{50} from 0.8 to 7 µM. Recently we have developed a *Leishmania* strain *L. (Leishmania)* NR resistant to GLIB (Gr) and have begun the identification of potential molecular differences between Gs and Gr. Therefore, in the present work we have evaluated the if the expression of resistance to GLIB, is correlated with changes in the expression of various proteins among them, P-gpA. Cultures of NRGs and NRGr were grown up to stationary phase, harvested and homogenized, subjected to PAGE-SDS, transferred into nitrocellulose sheets and immunoblotted against various antibodies. The results suggest that the development of resistance to GLIB does not change the expression of the paraxial flagellar proteins (B7) but, that the pattern of serine phosphorylated proteins differ among the two strains. Finally, antibodies related to multidrug resistance specific for human C-219 mdr and *L. tarentolae* glycoproteins GL-10 and GL-12, do not recognize specific peptides in Gr. Therefore, our results suggest that the expression of resistance to GLIB in *Leishmania* NR involves changes in the expression of serine phosphorylated proteins but is not related to the increase in the expression of proteins recognized by antibodies against human mdr or *L. tarentolae* glycoproteins.

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**CH-9**

**CHEMOTHERAPEUTIC EVALUATION OF IMIDAZOLIC DERIVATIVES ON EXPERIMENTAL CHAGAS’ DISEASE**

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In spite of having 6-8 million patients with Chagas’ disease in Brazil there is no effective drugs against the parasite. As such, we proposed to evaluate the action of imidazolic derived (benznidazole, itraconazole, fluconazole and benznidazole plus ketoconazole association) using three different strains. Non isogenic mice male, the Y, Romildo and Vicentina *T. cruzi* strains and the above related drugs were utilized. During the acute phase were realized, daily, parasitaemia study (Brener, 1962) and control of mortality up to negativation of all animals. In the chronic phase, during six months, were realized microhaematocrit and hemocultures monthly and then the survivors were sacrificed. Through the acute phase, test groups treated with benznidazole and with benznidazole plus ketoconazole association had an earlier negativation of parasitaemia, meanwhile control groups did not negativate. Remaining drugs conferred different outcomes. Lower levels of parasitaemia were obtained with test groups treated with benznidazole and benznidazole plus ketoconazole association in all strains. ketoconazole, fluconazole, itraconazole and benznidazole plus ketoconazole association when adapted separately resulted ineffective. The first revealed parasitaemia levels similar to those of the control group, while the other ones, revealed levels of parasitaemia higher than the control group when the Vicentina and Romildo strains were used. Itraconazole reduced the parasitaemia to levels comparable to benznidazole only when the Y strain was utilized. Mice inoculated with different strains and treated with benznidazole plus ketoconazole association revealed lower mortality. In summary, Romildo strain presented lowest mortality while the Y strain showed the highest. In the chronic phase the animals inoculated with different strains and treated with benznidazole plus ketoconazole association presented the lower percentage of parasitological positivity. The opposite occurred when were used fluconazole and/or itraconazole, which remained positive all along the experiment.
Through this study we assume that benznidazole and benznidazole plus ketoconazole association reduced the parasitaemia during the acute phase although mortality was only significantly reduced using benznidazole plus ketoconazole association. The other drugs showed different behaviors depending on the strain of parasite. In the chronic phase benznidazole plus ketoconazole association and benznidazole, respectively, presented higher efficient on the reduction of parasitaemia, but not totally. The other drugs were ineffective.

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CH-10
CHLORAMBUCIL: AN ALTERNATIVE DRUG TO ACUTE CHAGAS DISEASE TREATMENT
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Drugs currently available for treatment of Chagas’ disease diminish symptomatic acute phase, but because side effects and resistance to treatment, nitroderivates may not have satisfactory action. Thus, it is important the assay of alternative drugs to treated acute phase parasitaemia of Trypanosoma cruzi infection.

Chorambucil is an alkylating agent that promotes crosslinkage DNA files, preventing cell division, and acts also in RNA through formation of radicals that inhibit enzyme synthesis. Epimastigote and trypomastigote forms of T. cruzi (Y strain) were incubated with CLB. The largest trypanocidal effect have been observed with CLB 10^-3M, that reduced significantly the growth and viability of epimastigote culture forms, and also reduced viability of blood and culture trypomastigote forms.

To evaluate CLB effect in vivo, B10.A and BALB/c mice were infected (10^3 trypomastigote, Y strain, ip) and treatment was performed with 10 ml of drug (10^-4, 10^-5, 10^-6) Kg, administered ip in alternated days until death of all animals of the group. In B10.A mice we have similar increasing levels of parasitaemia until day 8 after infection, in treated and control groups. After 10 days of infection, animals treated with CLB 10^-4M showed clearly smaller levels of parasitaemia than untreated control mice. Mortality in B10.A mice started at day 12 after infection and did not show significant difference between treated and untreated groups. To BALB/c mice, parasitaemia of control untreated group was consistently upper than that of treated groups. Mortality in BALB/c mice started 10 days after infection and was significantly different of the mortality ratio showed in untreated control.

CH-11
COMPARATIVE ANALYSIS OF THE EFFICIENCY OF AMIDINES DERIVATIVES ON DIFFERENT ISOLATES OF TRYPANOSOMA EVANSI
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We have previously described the “in vitro” effect of N,N’-diphenyl-4-R-benzamidines (where R= H, OH, Cl, Br, CH$_3$, OCH$_3$ and CN) on a Trypanosoma evansi isolate derived from a horse. Based in other data from our group, related to the difference of the effectiveness of these compounds in the different evolutive stages of several trypanosomatids, we decided to compare the action of these compounds in other isolates of Trypanosoma evansi, which is the aethiological agent of “Mal de cadeiras”, a disease that is endemic in the the Pantanal Matogrossense area. The compounds were solubilized in DMSO and tested in a concentration range of 160µg/mL to 5µg/mL. In the present study the parasites were isolated from a dog and a coati, maintained in immunosupressed rats and further purified through a DEAE column. The trypomastigotes obtained were mixed to the different drugs’concentration, incubated at 25°C /24h and the remaining parasites counting in a Neubauer chamber. It is already known that these isolates have no difference from the biochemical and molecular point of view, however, concerning the sensitivity to the tested compounds some differences were observed. For instance, the methyl-derivative which was very effective against the horse isolate (LD50=14mM), had no effect on the coati and dog isolates (LD50=525mM and 195.5mM). Besides, the CN-derivative showed a good activity against the coati isolate and no effect on the horse and dog isolates (LD5s0=377mM and 481mM). These low sensitivity of the dog isolate to the tested compounds could suggest that these reservoir must be responsible for the maintainance of the disease in the cited area. Further studies must be done, in order to understand the difference observed among the isolates, concerning the effectiveness of the compounds.

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CH-12
CRUDE EXTRACT OF “CRAVINHO” [POROPHYLLUM RUDERALE (JACQ.) CASS.] SHOW ACTIVITY AGAINST LEISHMANIA (VIANNIA) BRAZILIENSIS AND LEISHMANIA (LEISHMANIA) AMAZONENSIS

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The treatment for leishmaniasis in Brazil has been basically with pentavalent antimonials, which have prolonged therapy and side effects. In this aspect, new chemotherapeutic agents have been tested. Thus, extract from plants of popular use in the treatment of leishmaniasis have been evaluated in relation to activity against Leishmania. The lyophilized crude extracts of “Cravinho” [Porophyllum ruderale (Jacq.) Cass.], with chlorophyll, were evaluated in vitro and in vivo for anti-leishmanial activity. For in vitro evaluation, promastigote forms of Leishmania (Viannia) braziliensis (MHOM/BR/87/M11272), cultivated in 199 (Difco) medium, at 25°C in log fase were tested with different concentrations of the extract. The parasites were counted in Neubauer’s Chamber to evaluate inhibition of growth. For each in vivo evaluation, were utilized three groups of hamsters. The groups I (Topic Treatment Group) and II (Positive Control Group) were infected on tail basis with promastigote forms of Leishmania (Leishmania) amazonensis (MHOM/BR/89/M12766) [experiment 1] and L. (V .) braziliensis [experiment 2]. The group III (Negative Control Group) was inoculated with sterile saline on the same place. For in vitro test, the better inhibition happened in the interval between 1.5 and 2.5 mg/ml, with 22.7%, 41.0% and 63.6% of the inhibition at 24, 48 and 72 hours of incubation. For in vivo tests, the groups I and III were treated for ninety days with a topic ointment containing crude extract at 10%. For in vivo experiment with L. (L.) amazonensis, among animals of Group I, just one showed ulcerated lesion and the other ones presented partial cicatrization, however with edema. In the Group II, everyone presented ulcerated lesions. With L. (V .) braziliensis, in five animals of the Group I, one of them has ulceration with diameter bigger than 9 mm, while among five animals of the Group II, three (60 %) presented ulceration on this aspect. These results show that the crude extracts in use have anti-leishmanial activity in vitro and decreased the lesions in vivo.

Supported by CNPq, UEM

CH-13
EFFECT OF BENZNIDAZOLE IN VIVO AND IN VITRO ON STRAINS OF TRYPANOSOMA CRUZI ISOLATED FROM WILD RESERVOIRS AND TRIATOMINES FROM PARANA, BRAZIL

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Strains isolated from T. cruzi from different regions or hosts can present interspecies variations. Currently Benznidazole is the only commercially available drug, although clinical trials reveal that its activity is only partial. In the present study we evaluated the in vivo sensitivity of Benznidazole in T. cruzi strains isolated from triatomine (G3) and wild reservoir opossum (A2.1A). In addition, we studied the in vitro action of Benznidazole on the mitochondrial oxidative metabolism of the same strains. For in vivo study, groups of 30 mice were used which were inoculated with 1x107 metacyclic trypomastigotes. The animals were then divided into 2 groups of 15; one group was treated with Benznidazole for 20 consecutive days and the other did not receive the drug. For the in vitro study, trypanosomes isolated from the wild reservoir and triatomines were cultivated in LIT medium until reaching cell density of 3x10⁸ cells/ml. The cultures were then centrifuged and the resulting pellet was submitted to the mitochondrial isolation technique described by Braly et al. (1974). The lysate was adjusted with sucrose, the cellular remains were removed by centrifugation, the pellet was resuspended in wash medium and centrifuged. Shortly thereafter, polarographic measures of oxygen consumption with and without Benznidazole were done. In vivo,G3 was sensitive to Benznidazole (85% cure) and A2.1A strain was resistant (23% cure). The in vitro study revealed inhibited basal respiration of the mitochondrial in relation to succinate and glutamate.

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EFFECT OF TERPENES IN CULTURES OF *PLASMODIUM FALCIPARUM*

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Resistance of *P. falciparum* to most available antimalarials is widespread and the development of new drugs is regarded as a priority. Recently, we have demonstrated the presence of an active isoprenoid pathway in the intraerythrocytic stages of *P. falciparum*. Parasites treated with mevastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-Co A) reductase inhibitor, decreased the biosynthesis of dolichol and dolichyl phosphate in the ring stage. This fact was correlated with a concomitant arrest of parasite development “in vitro”. Therefore, inhibition of dolichol biosynthesis or competition with similar molecules, as terpenes, could identify new targets for the development of new anti-malarial drugs.

We evaluated the activity “in vitro” of 3 terpenes, nerolidol, farnesol and linalool, through the parameter IC\textsubscript{50}/48 hours compared with mefloquine, which is currently used in malaria therapeutics. Values of IC\textsubscript{50} of nerolidol farnesol and linalool against *P. falciparum* isolate S20 were 30 µM, 36 µM and 380 µM respectively, nevertheless mefloquine was 28 nM. Nerolidol and farnesol were tested against two other isolates (*P. falciparum*, PA and *P. falciparum*, RP), showing similar IC\textsubscript{50} values.

Biosynthesis of glycoproteins was inhibited in ring, trophozoite and schizonts stages after nerolidol and farnesol treatment of parasite cultures. These facts were correlated with a concomitant arrest of parasite development. We cannot exclude the fact that other end products of this pathway such as isoprenylated proteins and/or ubiquinones may also be inhibited by this treatment. Therefore, the isoprenoid pathway may represent a different approach for the development of new antimalarial drugs. Studies to address these questions are currently in progress in our laboratory.

Supported by Fapesp, UNDP, World Bank, WHO

EFFECT OF TREATMENT WITH TRYPANOCIDAL NITRODERIVATIVES IN THE PROGNOSIS OF CHAGAS’ DISEASE PATIENTS NOT SUBJECTED TO EXOGENOUS SUPERINFECTIONS

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We have provided medical care to the street-cleaners of Brasília, Federal District. Among 718 street-cleaners from Minas Gerais (37.2%), Goiás (34.1%), Piauí (7.7%), Bahia (6.9%), Maranhão (3.1%) and other Northeast States (2.3%), were found 18.6% with four immunological exams positive for *Trypanosoma cruzi*. A cohort of these chagasic individuals had already been treated with the nitroderivatives Nifurtimox or Benznidazole, before the health programme was initiated. In this study, we report clinic and laboratory data of 45 treated chagasic patients, 46 untreated chagasics, during 10 years of observations. The control group consisted of 41 individuals showing negative immunologic exames for the infection. All 132 males in this study were matched by age.

Xenodiagnoses were carried on in each chagasic individual, and the parasite was recovered in three treated and in five untreated cases. PCR amplification products were obtained from the DNA of buffy coat cells of the blood of each chagasic patient, regardless of being treated or not-treated, when *T. cruzi* kDNA and nuclear DNA primers were used.

Five ECG recordings were obtained from each individual at several occasions, and ventricular premature beats, bundle branch blocks and intraventricular conducting disturbances were analysed. All these alterations were seen five to eight folds more frequent in the chagasic individuals than in the controls. The premature beats were three folds more frequent in the untreated than in the treated chagasic patients. On the other hand, intraventricular conducting disturbances were twice more frequent in the treated chagasics than in the untreated ones, during the same period of observation. The 24 h recordings (Holter) revealed ventricular arrhythmias in 62.5% of the treated chagasics, in 58.3% of untreated patients and in 23.5% of the controls. The ecocardiogram displayed alterations in 33.3% of treated and in 28.1% of untreated chagasics, and in 25% of the controls. Of interest, the ecocardiogram showed aneurismal dilation of the left ventricle in three untreated chagasics only. During the period of observation, three untreated and three treated chagasics died.

Supported by FAPDF
CH-16  
EFFECTIVENESS OF TRICYCLIC DRUGS FOR TRYPANOSOMA CRUZI INFECTION TREATMENTS MEASURED BY CARDIAC RECEPTORS FUNCTION

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We have previously demonstrated that cardiac receptors’ function is altered along the acute, indeterminate and chronic phase of experimental Chagas’ disease, considering this parameter as a good and early detector of this pathology. In the present work we used cardiac receptors’ affinity and density to evaluate the effectiveness of some tricyclic drugs used in clinics (Thoridazine (THI) and Clomipramine (CLO)) as therapy of T. cruzi infected mice. Albino Swiss mice (n=160) were intraperitoneally inoculated with 50 trypomastigotes/mouse of T. cruzi, Tulahuen strain. The infected animals were divided in (a) without treatment, (b) treated with THI 80 mg/Kg for 3 days oral way, (c) treated with CLO 40 mg/Kg injected intraperitoneally 1 hour and 7 days post infection. A non infected group, control, was also analyzed. β-receptors density (78.2 ± 1.67, 77.28 ± 0.91 and 54.1 ± 4.3 fmol/mg.prot) and affinity (5.63 ± 0.26, 6.85 ± 0.20 and 11.7 ± 1.58 nM) were determined in the 3 phases of T. cruzi infection by binding of dihidroalprenolol trited were different when compared with control (71.96 ± 0.36 fmol/mg.prot and 3.60 ± 0.050 nM). THI and CLO treatment modified β receptors function turning density and affinity was similar to non infected since 75 days p.i. until chronic phase. Besides histopathological studies of heart showed mononuclear infiltrate without amastigotes at 35 days p.i. None structural alterations were detected in chronic phase. Present results showed that both tricyclic drugs prevented a permanent damage in cardiac receptors function that would be the beginning of the chagasic cardiopathy.

Support: SECyT, Conicor.

CH-17  
EFFICACY OF TREATMENT OF CUTANEOUS LEISHMANIASIS WITH INTRALESIONAL HUMAN RECOMBINANT GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR (hrGM-CSF) COMBINED WITH ANTIMONIAL

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Pentavalent antimonials are standard treatment of Cutaneous Leishmaniasis (CL). However, there are associated complications, such as: daily iv injections; adverse reactions; delayed treatment response. GM-CSF has the ability in vitro to kill leishmania and has been used to treat ulcers of different etiology. The objective of this work was to evaluate the effect of intralesional rGM-CSF as adjuvant therapy in reducing the healing time of ulcer. Twenty CL patients were selected for a double blind randomized trial. Ten patients received 2 intralesional doses of 200 mg of rGM-CSF at a week interval and antimonial therapy (20 mg/Sb v/kg of body weight per day, during 20 days, iv). The control group received intralesional saline instead of rGM-CSF. The patients were evaluated at a 30 days interval for 6 months and healing criteria was total scar of the ulcer. Results: The GM-CSF group had significantly increased cure of the ulcers at 40 days in comparison to the control group (RR 7.00, 95% CI 1.04 to 46.97; p<0.05). Although not significant, the GM-CSF group had low cases that require retreatment with antimonial (2/10 vs 4/10). There was no difference in the following parameters: age, size, duration of disease and Montenegro skin test. The mean ± SD of healing time of the rGM-CSF group 49±38 days and control group was 110±62 (p=0.02). GM-CSF increased the levels of IFN-γ from PBMC cells and also lymphoproliferative response. This study demonstrates that intralesional rGM-CSF given as adjuvant therapy with antimonial reduces the time for cure from cutaneous leishmaniasis ulcer, that can be mediated by immune response and also by the ability of GM-CSF to induce scar formation.

Supported by Finep, CADCT, Pronex.

CH-18  
EVALUATION OF ANTI-TRYPANOSOMAL ACTIVITY OF SEVERAL ANALOGOUS FROM MEGAZOL

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Recent reports from the World Health Organization describe that in South America about 20 million people are infected by Trypanosoma cruzi. Megazol [2-amino-5(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole] is a broad
spectrum anti-bacterial and anti-parasitic compound. The drug has also shown a marked curative effect in experimental Chagas’ disease, being effective against strains that are resistant to the clinically employed agent nifurtimox and benznidazole (Tsuhako et al., Bio. Pharm. 1989, 38(24),4491). We have investigated the chemical constitution of megazol and generated megazol compounds based on new substitution principles. This work report four new megazol derivatives, substituted at position 1 of the imidazole ring and position 2 at the thiadiazole ring. Position 1: Mega-S - [2-amino-5(1-ethylthioethyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole]. Position 2: Mega-G - [2-ethoxy-5(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole]; Mega-T - [2-thiourea-5(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole]; Mega-A - [2-(4-carboxybutamide)-5(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole].

Different from megazol, none of these compounds present toxicity to sheep blood cells. We evaluated the anti-tripanosomal activity of these analogous on trypomastigotes cultures of Y strain and cell viability was determined after 24 and 48h. Megazol and the megazol compounds T, S, A were 100% effective on anti-tripanosomal activity after 24h, while the compound G show no activity. In conclusion, we have generated megazol derivatives with similar anti-tripanosomal activity but lower cell toxicity compared to megazol.

CH-19
EVALUATION OF GUANYL HIDRAZONES AS A TRYPANOCIDE TO CONTROL PARASITE TRANSMISSION BY BLOOD TRANSFUSION

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Blood transfusion has been recognized as having an increasing important role in the transmission of Chagas’ disease in Latin America. A chemoprophylaxys approach to this problem has been the use of several chemicals. A series of 26 aromatic mono-guanyl hidrazones with different substitution patterns were prepared from the respective aldehydes by Dean-Stard promoted condensation with aminoguanidine hydrochloride. All the products were completely characterized using spectroscopic techniques, mainly 1H and 13C nuclear magnetic resonance (NMR) and high resolution mass spectroscopy. In this work the effect of the synthesized compounds against T. cruzi (Y strain) was investigated using trypomastigote forms (2-5 x 10^6 cells/mL) in presence of blood. Untreated and gencian violet-treated parasites were used as controls. Cell counts were performed after 24h at 4°C and the drug concentration corresponding to 50% parasite elimination was expressed as the ID_{50}. The more active compound showed ID_{50} 0,25 mg/mL.

<table>
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<th>R1</th>
<th>R2</th>
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<th>ID_{50}(mg/mL)</th>
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<td>OCH_3</td>
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<td>H</td>
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<td>OCH_3</td>
<td>0,30</td>
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In the same experiment condition, gencian violet showed an ID_{50} of 0,30mg/mL. From these results we can conclude that the substitution in the benzene ring of aromatic guanyl hidrazones with halogen and methoxi groups led to a lytic activity against T. cruzi. Linear correlation’s (QSAR) between trypanocide activity and electronic effects of the substitutess has been observed.

Supported by Capes.
**CH-20**

**EVALUATION OF THE IN SITU IMMUNOFLUORESCENCE ASSAY: A FOLLOW-UP STUDY OF PATIENTS WITH CHRONIC CHAGAS DISEASE SUBMITTED TO CHEMOTHERAPY**

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The *in situ* indirect immunofluorescence assay (ISIFA) was developed for discriminating treated from nontreated chronic chagasic patients (J.Clin.Lab.Anal 10:98-103, 1996). Successfully treated patients showed IgG antibody titers ranging from 40 to negative, whereas nontreated patients showed titers varying from 80 to 320. In this work, the decay of antibody profiles was investigated by ISIFA, following-up 100 chronic chagasic patients before and after the specific treatment performed with benznidazol. Serum samples were collected before treatment, right after the treatment and at least two more samples in a period of 2 years. Besides ISIFA, conventional serological assays and xenodiagnosis (XENO) were carried out. The obtained data permitted to divide patients into four groups: A: (57%): patients showed significant antibody decay, corresponding to more than two titers, and some of them presented completely negative results; B: (30%) patients showed no significant antibody decays; C: (11%) patients whose results oscillated and nowadays present titers considered positive and D: (2%) patients who showed no antibody decay and positive XENO. In contrast, all the other groups presented negative XENO after treatment during the period of this study. In 76.6% of patients from group A had significant antibody decay within a period of 3 years post-treatment, however 57.3% of them had such decay in two years after treatment. Taking together the data provided by ISIFA and XENO we think that the treatment was efficient in patients from group A, whereas those from group D had therapeutic failure. Patients from group B and C, are being submitted to several parasitological tests (Xeno or hemoculture) repeated in different times intervals, in order to confirm the data provided by ISIFA.

**CH-21**

**FURTHER STUDIES ON THE EFFECT OF ALKYL LYSOPHOSPHOLIPIDS ON TRYPANOSOMA CRUZI**

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Giving continuity to a study about the antitrypanosomal activities of alkyllysophospholipids (ALPs) (Santa-Rita et al., *Mem.Inst.Oswaldo Cruz* 92(supl):326,1997), we analysed the effect of edelfosine (Ed), ilmofosine (Ilm) and mitelfosine (Mit) on the proliferation of T. cruzi. The values of ED_{50} (nM) are displayed below:

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<thead>
<tr>
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<th>epimastigotes</th>
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<td>h</td>
<td>24</td>
<td>48</td>
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<td></td>
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<tr>
<td>Ed</td>
<td>11.4±0.2</td>
<td>6.5±1.3</td>
</tr>
<tr>
<td>Mit</td>
<td>26.6±3.7</td>
<td>9.3±0.3</td>
</tr>
<tr>
<td>Ilm</td>
<td>17.4±1.2</td>
<td>9.2±0.0</td>
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</tbody>
</table>

To measure intracellular calcium levels the parasites were loaded with fura-2, resuspended in PBS, and the ALPs (2.5 to 15 nM) was added directly into the cuvette. The effect of the drugs on T. cruzi was more pronounced in absence of serum (PBS) as already described for tumoral cells (Lohmeyer & Workman, *Biochem. Pharmacol*. 45:77,1993). Increase on calcium levels on the three forms of the parasite was associated with a permeabilizing action of the drugs.

The treatment of the parasites with ALPs caused several morphological modifications observed by electron microscopy, such as cell swelling, disorganization of the inner mitochondrion membrane and more characteristic alterations on the flagellum membrane with strong vesiculation.

The pre-treatment of epimastigotes for 1h at 37°C with 30 nM Ed followed by washings and resuspension in DMES at the same concentration as non-treated parasites, caused an inhibition of the infection of heart muscle cells (HMC) above 40% after 4 h of interaction. Maintaining the interaction parasite-host cell for more 20 h, it was observed a progressive reversal of this inhibition. Twenty four hours after removal of non-interiorized parasites, the inhibition of the infection was in the range of 30 to 60% for drug concentration between 15 and 60 nM.

Supported by EC (INCO-DC IC18-CT96-0084), CNPq, Papes, Fiocruz.
GROWTH INHIBITION AND ULTRASTRUCTURAL CHANGES INDUCED BY LICHENIC SUBSTANCES ON LEISHMANIA CHAGASI IN VITRO

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Leishmaniasis is an increasing public health problem in Brazil. The only drug of choice for both visceral and cutaneous leishmaniasis is sodium stibogluconate (Sb V), a 40 years old drug. Although resistant Leishmania strains are still rare, there is a growing number of cases were the conventional treatment fails. On the other side, compliance to i.m. Sb V treatment in cutaneous cases is rarely achieved, specially in rural areas, far from the medical continuous surveillance. New drugs that could be used either as topic unguents or orally are needed. In this work we describe the action of six lichen substances on Leishmania chagasi, the etiological agent of kala-azar in the Americas. The following substances were tested: fumarprotocetaric, protocetaric, secalonic, hypostitic and difractaric acids and atranorine. Density readings were obtained daily from promastigote cultures on LIT medium, at 26 °C, containing up to 40 µg/ml of one of the substances. A >50% growth inhibition was observed only when atranorine and difractaric acids were used at concentrations of 30 and 40 µg/ml, atranorine being always more effective than difractaric acid. Promastigotes from atranorine- and difractaric acid-containing cultures and from control cultures were fixed with glutaraldehyde and prepared for transmission electron microscopy by standard techniques. Mielin whorls in abnormal mitochondria were observed in many cells treated with atranorine, in both concentrations, and in some of the promastigotes from difractaric acid-containing cultures, but was rarely observed in control cultures. Other cell structures were apparently not affected. Our results points toward those two lichen substances as potential new drugs in leishmaniasis.

GROWTH INHIBITION OF LEISHMANIA (LEISHMANIA) AMAZONENSIS BY AUSTRALIAN SNAKE VENOMS

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Cutaneous leishmaniasis is an endemic disease, mostly found in tropical and subtropical regions. In Brazil, it is registered 26000 new cases/year considering all their clinical forms. The actual treatment is based on some toxic drugs such as pentamidine and antimoniais. The aim of this project is to determine Australian snake venoms with anti-Leishmania activity on Leishmania (Leishmania) amazonensis. Acanthophis antarcticus, Agkistrodon bilineatus, Hoplocephalus stephensi, Naja melanoleuca, Notechis ater niger, Notechis scutatus, Oxyuranus microlepidotus, Oxyuranus scutellatus, Pseudechis australis, Pseudechis colletti, Pseudechis guttatus, Pseudechis porphyriacus and Pseudonaja textilis venoms were incubated at the concentration of 40 µg/mL in 25mM EDTA with 3,0x10^6 parasites/mL. Promastigotes from L.(L.) amazonensis were grown in RPMI 1640 medium without phenol red plus 10 % fetal bovine serum at 25 °C. The parasites viability was measured by a colorimetric assay based on the conversion of MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (5mg/ml) in colored (570nm) formazan.

A. bilineatus, H. stephensi N. melanoleuca, P. australis, P. colletti, P. guttatus and P. porphyriacus venoms showed anti-Leishmania activity and even after irradiation with 2000 Gy dose, they still kept their inhibition growth activity. The L-amino acid oxidase (LAO) activity was tested in all venoms, using RPMI as substrate and peroxidase and OPD (o-Phenylenediamine) as revealing reagents. The same venoms, including the irradiated one, that presented the anti-leishmania activity, showed LAO activity. These data suggest that the anti-leishmanial effect could be related by L-amino acid oxidase activity.

Supported by CNPq, Rhae, LIMHCFMUSP.

IMMUNESUPPRESSION REDUCES THE ACTIVITY OF STEROL BIOSYNTHESIS INHIBITORS (SCH5692 AND DO870) BUT NOT BENZNIDAZOLE (BZ) IN MICE ACUTELY INFECTED WITH TRIpanosoma CRUZI

The sterol biosynthesis inhibitors (SBI) have been identified as potent in vitro antiproliferative drugs against *T. cruzi*. We have recently demonstrated that the SBI SCH56592 and DO870 were more effective in vivo against resistant *T. cruzi* strains than Bz, the currently used drug in the treatment of Chagas’ disease. In order to evaluate whether the effectiveness of SBI on *T. cruzi* strains was dependent of the cooperative action with the host immune system, we have evaluated their curative activity on immunesuppressed mice. Adult Swiss females mice were immunesuppressed with cyclophosphamide (Cy) two days prior *T. cruzi* infection. The mice were infected with *T. cruzi* strains presenting different susceptibilities to Bz: CL (susceptible), Y (median resistant) and Colombiana and SC28 strains (resistant). Animals were submitted to 20 days of treatment with oral doses of SCH56592, DO870 and Bz, starting at day four after infection. Thirty to 60 days after the end of treatment, animals were initially evaluated through parasitological methods and further using immunological approach, in order to monitor the percentage of cure. Hemoculture (HC) and xenodiagnosis (XD) were chosen as parasitological methods to identify the presence of parasites. Under negative HC and XD results, the immunological status of individual serum was addressed through the analysis of anti-live trypomastigote antibodies by flow cytometry. Our results demonstrated that immunesuppression reduce host life span the infected mice regardless of the treatment. However, treatment with SBI increased survival in comparison to Bz in animals infected with CL, SC-28 and Colombiana strains, even in immuno competent mice. SCH56592-0 was more efficient than DO870 to prevent death in immunesuppressed animals infected with Y strain, with no effect on other strains. Immunesuppression reduces the activity of SBI but not Bz in mice infected with Y, Colombiana and SC-28 strains. No effect induced by immunesuppression was observed in animals infected with CL strain. The trypanocide effect of SBI was drastically reduced by immunesuppression in animals infected with Y and SC-28 strains, with little or no effect on animals infected with Colombiana and CL strains, respectively. In conclusion our results showed that the efficacy of treatment of Chagas’ disease depends on the drug, the *T. cruzi* strain and the host immune system. Analysis of anti-live trypomastigotes antibodies showed that their disappearance is a later event than parasite clearance, indicating that its use as a cure criteria would be applied only over 3 months after treatment.

Supported by Conicit, Papes, Fiocruz, Pronex.

**CH-25**

**IN VIVO AND IN VITRO EVALUATION OF TOXICITY OF AZORELLANOL, A NATURAL PRODUCT WITH TRYPANOCIDAL ACTIVITY**

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We have previously reported the trypanocidal activity Azorellanol, a new diterpenoid isolated from *Azorella compacta* (Umbelliferae). In order to know aditional information about this trypanocidal molecula, we evaluate the effect of Azorellanol against testicular function. Balb/c mice were treated with Azorellanol at day 0 and every 5 days during a period of 45 days using a concentration of 50 mg kg⁻¹ by mouse. In each mouse the spermatogenic and esteroigenic activity were evaluated. Simultaneously, cytotoxicity assay were performed using against HeLa and Vero cells.

No differences were observed between experimental and control mice when parameters as body weight, testicle weight and epididimal weight were evaluated. Histological analysis of testicles did not shown any alteration. The lethal dose 50 (LD₅₀) for HeLa and Vero cells was 0.139 mM and 0.132 mM, respectively.

This preliminary finding suggest that Azorellanol, a new trypanocidal natural products, could be atoxic for mammalian cells. On the other hand, we propose the in vivo evaluation of testicular function as criteria for toxicity evaluation of natural compounds with trypanocidal activity.

Support: Fondecyt, Chile.

**CH-26**

**IN VIVO EFFECT OF BENZNIDAZOLE TREATMENT ON THE SURFACE OF DRUG-SUSCEPTIBLE AND RESISTANT TRYPANOSOMA CRUZI POPULATIONS**

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*In vivo* effect of benznidazole (BZ) treatment on the surface of drug-susceptible and resistant *T. cruzi* populations was investigated. The populations of *T. cruzi*, Y strain, wild-type susceptible (WT) and resistant to BZ (RES), previously selected in *in vivo* (Murta & Romanha, Parasitol. 1998, 116: 165-171) were used. Mice infected with either parasite populations were treated at the peak of parasitemia with a single high dose of 500mg of BZ/kg of body...
weight. Trypomastigotes isolated from untreated infected mice, as well as, 3h after treatment with BZ were evaluated at the molecular level, using flow cytometry. Total protein content (PC) was evaluated by the binding of fluorescein isothiocyanate (FITC); membrane-bound immunoglobulins (Ig) by FITC-conjugated anti-mouse IgG antibodies, and phosphoglycoprotein (PGP) expression by indirect immunoassay using anti-PGP monoclonal (C219) as the primary antibody. The results showed that BZ treatment lowered the level of PC on the WT cell surface, whereas it had no effect on the RES population when live parasites were treated with FITC. These data suggest that either the treatment eliminates some parasite surface proteins or the WT parasites promptly shed the protein-FITC complex from its surface. Treatment decreased the amount of membrane-bound Ig in RES population, an to a greater extent in the WT population. Chronic mouse sera bound equally to WT and RES parasites isolated from irradiated mice, independent of previous treatment. A high level of PGP expression has been associated with drug-resistance phenotype in several protozoa (Ullman, J. Bioenerg. Biomemb. 1995, 27:77-84). We observed a higher level of PGP in RES T. cruzi than WT susceptible parasites. BZ treatment increased the PGP level in RES parasites and decreased PGP level on WT ones. Our data suggest that BZ eliminates some proteins and/or down-regulates the protein expression on membranes of WT parasites with almost no effect on RES parasites. Moreover, these results suggest that the resistance to benznidazole treatment could be correlated with the ability of parasites to up-regulate the PGP expression under drug pressure.

Supported by CNPq, Fapemig, Papes,Fiocruz, Pronex.

**CH-27**

**INTERACTIONS STUDIES OF GUANYLHYDRAZONES WITH IONIC MICELLES AS TRYPANOSOMA CRUZI MODEL MEMBRANES**

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We recently showed that aromatic guanylhydrazones are promising anti T.cruzi agents. Most of the compounds, were significantly more active than crystal violet (ID50 536mM), when tested in vitro against the trypomastigote form of Trypanosoma cruzi. Molecular modeling study of these compounds with B-DNA showed that the drugs clearly preferred interaction with A T rich regions of the DNA. The mechanism of action of these compounds is not yet know, neither the molecular mechanisms of guanylhydrazones interaction with T.cruzi membranes.

![Figure I: GH= -C=NHNHC=(NH 2 ).HCl](image)

In this work, we report the study of T.cruzi interaction between guanylhydrazones, Figure I, and membranes, using micellar systems as mimic models. The study was performed using SDS and CTAB micelles. NMR techniques, spin-lattice relaxation time, $^1H$ T1, NOE and NOESY, allowed us show that the guanylhydrazones with higher affinity to the anionic micelles (SDS) are also to more active against T.cruzi. The least active compound does not differentiate between anionic and cationic (CTAB) micelles. Although these are only preliminary results, they reveal a direct relationship between the ability of interaction of these compounds with the anionic micelles and degree of activity.

Supported by PADCT, Finep.

**CH-28**

**INVESTIGATION OF HUMORAL MARKERS FOR THE FOLLOW-UP OF EXPERIMENTAL CHAGASIC MYOCARDITIS IN MICE UNDER CHEMOTHERAPY**

De Souza AP, Olivieri BP, Cotta-de-Almeida V, Oliveira GM, Batista MM, Gomes FHM, Henriques-Pons A, De Castro SL, Araújo-Jorge TC
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Tissue enzymes in blood are indicators of tissue lesions in Chagas' disease (Exp.Parasitol.40:411,1976; Comp.Biochem.Physiol.64B:11,1979). We analysed if creatino phosphokinase (CK) and its specific heart isoenzyme CKMB could indicate myocarditis in C57BL6 and Swiss T. cruzi-infected mice (Y strain), and compared them with animals treated with Benznidazole (Bz). Non-infected mice samples (n=282) allowed the definition of normal ranges. A cut-off was calculated with the mean+2sd, to which values of infected samples were referred. Plasma collected from mice infected with $10^4$ parasites showed a CKMB increase at the 2nd week post-infection (wpi) in 41.6 and 15.4% of Swiss and B6 mice, respectively. CK levels increased as CKMB. Infection with $10^2$ did not increase CKMB and allowed the survival of mice which could then be followed during the chronic phase. 80.9% and 3.7% chronic Swiss and B6 mice showed increased enzyme levels, respectively. Mice with high CKMB levels submitted to an activity test using running wheel showed low performance. Mice groups were named: infected & treated (Group IBz), infected & non treated (Group I), non-infected & non-treated (Group N). Schemes were: (1) infection with $10^4$ parasites plus abortive treatment per os with 100mg/Kg Bz for 9 consecutive days; (2) infection with $10^2$ parasites plus Bz treatment in drinking water (0.25 mg/ml) for 50 days; (3) infection with $10^5$ plus Bz in drinking water at 0.10 and 0.25mg/ml for 15 days starting at the 2nd wpi. In Schemes 1 and 2, IBz mice presented no detectable parasitemia nor mortality. Scheme 1 gave 100% cumulative mortality (%CM) in I mice, which dropped to 40% in Scheme 2. In Scheme 3, IBz showed 70 and 40 %CM respectively for 0.1 and 0.25mg/ml. Bz in Scheme 1 avoided the splenomegaly and the increase of enzyme levels which were detected in I group at the 2nd wpi. However, in the 3rd week, when splenomegaly was reduced both in I and IBz, 26.3% of IBz mice had high CKMB levels. Thymic atrophy was clear in the third wpi in the I group and was reversed by IBz, but lymphoadenomegaly observed at the 2nd and 3rd wpi was not reversed by Bz. Scheme 2 did not avoided CKMB increase in chronic mice, but frequency of mice with high CKMB levels from 80.9% to 51.3% in IBz. Heart histopathology results will be shown. The results suggest that CKMB can be used as a tracer of active chronic myocarditis and that Bz treatment reverts only partially heart damage.

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CH-29
LEISHMANIA AMAZONENSIS: IN VIVO EXPERIMENTS WITH DIARYLHEPTANOIDS FROM LEGUMINOSAE AND ZINGIBERACEAE PLANTS

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In a previous work we demonstrated that diarylheptanoids extracted from Centrolobium sclerophyllum (Leguminosae) are very active against Leishmania amazonensis promastigotes. In order to continue our studies with these class of compounds, we decided to evaluate the activity of curcumin, a phenolic diarylheptanoid derived from Curcuma longa (Zingiberaceae), against the extracellular forms of L. amazonensis Chemical modifications were done in the structure of the curcumin molecule, in order to increase the activity and we found that the most active compound was the methylated derivative. In this work we are now showing our in vivo results with the following diarylheptanoids derivatives: des-o-metilcentrolobine (LD50=57mM), the most active compound extracted from C. sclerophyllum and the methylated curcumin (LD50=5mM). In the in vivo experiments several groups of Balb/c mice were injected subcutaneously with 3x10^6 parasites/50mL. The drugs were injected in a concentration of 20mg/Kg weight, following different schedules, such as: a) only one injection of each drug, one week after the infection; b) two injections of each drug, being the second injection made after 15 days of the first dose; c) two injections of each drug, being the second dose given 45 days after the first dose. One month after the infection, we iniciated the measurements of the lesions up to 75th day. The results showed that when only one dose of the compounds was applied, the mice treated either with the des-o-methyl centrolobine or methyl curcumin, at the 45th day of following up, showed a decrease of 34.5% and 55.5% in the lesion size, respectively, when compared with the mice inoculated with the parasites alone.

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CH-30
L-AMINO ACID OXIDASE ACTIVITY FROM BOTHROPS MOOJENI VENOM ON LEISHMANIA (LEISHMANIA) AMAZONENSIS PROMASTIGOTES

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Cutaneous leishmaniasis is a tropical disease found mainly on endemic areas as Amazon and Northeastern of Brazil. It causes a disfiguring skin disease that is resistant to most treatments, except to those using toxic antimonial salts. The need of development of new drugs is eminent. Snake venoms have been reported as a strong inhibitors of
protozoas, but no attempts to define the active fraction were done. In this report, we analyzed the anti-Leishmania activity of the crude venom and the purified fraction (L-amino acid oxidase -LAO), from Bothrops moojeni venom, on *L. amazonensis* promastigotes, using an *in vitro* assay. The Efficient Concentration 50% (EC_{50}) of the crude venom was determined, using the same method, resulting in about 2mg/mL. The purification procedure was followed by molecular exclusion chromatography and ion exchange chromatography, in a FPLC system. A fast procedure of detection of LAO activity, was developed by Venom Group-IPEN, using in a colorimetric assay, RPMI-PR - 1640 as substrate and peroxidase and OPD as a revealing reagents. *L. amazonensis* was grown as promastigotes in RPMI-PR 1640 with 10% fetal bovine serum, at 25°C. The viability of promastigotes was detected by respiratory oxidative conversion of MTT in a colorimetric assay. This preliminary data, suggest that this venom had an active action against these parasites, and if adequately tested, could be used as an alternative therapy for cutaneous leishmaniasis.

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**CH-31**

**LEISHMANICIDAL ACTIVITY AND ULTRASTRUCTURAL CHANGES INDUCED BY SULPHUR SYNTHETIC NEOLIGNAN ANALOGUES**

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Leishmaniasis is a disfiguring and sometimes fatal protozoan disease affecting over 12 million people worldwide, and for which there is still no safe vaccine. Recently, a dramatic increase in the rate of Leishmania infections in HIV patients, together with the development of drug-resistance by the parasites has worsened this problem. Despite the tremendous progress made in the understanding of the biochemical and molecular biology of the parasite, the first-choice treatment for the several forms of leishmaniasis still relies on daily intramuscular injections of pentavalent antimonials developed more than 50 years ago. Therefore, the search for novel, effective and safe therapeutic compounds has become a priority. We have previously demonstrated the strong activity of the synthetic sulphur neolignan analogue LS-SCI against *L. amazonensis* (IC_{50}=1mg/ml). In this study, the ultrastructural changes induced by LS-SCI in *Leishmania amazonensis* and the activity of its analogues on *L. donovani* was studied. Peritoneal macrophages were infected with *L. donovani* and cultivated for 48 h with 15 LS-SCI synthetic analogues at 80 ng/ml. At the end of culture, the number of intracellular parasites was determined. Out of all substances tested, only LS-SCI was active, decreasing the intracellular parasite load by 94 %. To investigate the ultrastructural changes produced by LS-SCI on the parasites, *L. amazonensis* promastigotes were cultivated with 50mg/ml LS-SCI for 48 h and then processed for electron microscopy. LS-SCI induced the development of a very large vacuole in the parasite and disruption of its kinetoplast. These results demonstrate that the antileishmanial activity of LS-SCI is not restricted to the cutaneous *L. amazonensis*, but is extended to other parasite species, such as the visceral *L. donovani*. The various molecular changes made on LS-SCI did not further improve its activity, indicating that its core sulphur structure is the most active and least toxic.

**CH-32**

**MECHANISM OF ACTION OF N,N-DIMETHYL-2-PROPEN-1-AMINES ON TRYPANOSOMA CRUZI EPIMASTIGOTE FORM**

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The ergosterol biosynthesis has been largely studied as a target in the development of new antifungal and antiprotozoal agents. A preliminary study showed that 3-(4'-Bromo[1,1'-biphenyl]-4-yl)-3-X-phenyl-N,N-dimethyl-2-propen-1-amines act on sterol synthesis form *T. cruzi* epimastigote form (De Conti et al. XXIII Reunião Anual da SBBq, Maio 1994, Abstr. O1 (1993)). The non-substituted derivative of this series demonstrated (Oliveira et al. Mem Inst. Oswaldo Cruz 91, 320 (1996)) also activity on *Trychophyton rubrum* (5507 CCT strain), inhibiting the ergosterol synthesis from this dermatophyte (Oliveira et al. Mem Inst. Oswaldo Cruz 92, Sup1.1, 524 (1997)). More recently, the investigation of trypanocidal mode of action of this compound was continued using *T. cruzi* epimastigote form. The culture was maintained in LIT medium supplemented with 10% of FBS. When the density reach 10^7 epimastigotes/mL the parasites were exposed to the drug (12 nmol/L in DMSO) at two different periods (24 and 48 hours). After classical extraction procedure, the neutral lipids fraction (control and treated samples) was analyzed by HPLC and GC-MS. The chromatographic studies indicate reduction of ergosterol levels and increasing of squalene levels in the treated forms after 48 hours of exposition. Based on this preliminary results we can conclude that this new series of compounds act as inhibitors of ergosterol biosynthesis from *T. cruzi* epimastigotes.

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MODELLING OF THE IRON SUPEROXIDE DISMUTASE FROM TRYPANOSOMA CRUZI: AN ALTERNATIVE FOR THE TREATMENT OF CHAGAS' DISEASE

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Superoxide dismutase (SOD) is a metalloenzyme which plays an important role in oxidative defense in most organisms to remove excess superoxide radicals via dismutation to oxygen and hydrogen peroxide. SOD is widely distributed in animal tissues and has also been found in several parasites.

The epimastigotes of Trypanosoma cruzi, as well as most trypanosomatids, have poor enzymatic defenses against intracellular hydroperoxides. Catalase and Se-containing Glutathione peroxidase are absent and Superoxide dismutase has been detected in relatively low proportion as compared to mammalian cells.

Trypanosoma cruzi enzymes involved in antioxidant defense may be of importance in the context of improved chemotherapeutic approaches because a number of chelating agents and some of their derivatives have been as effective as, or superior to, benznidazole, the compound currently in clinical use, in the suppression of the reproduction of epimastigotes of Trypanosoma cruzi.

In this work, molecular modelling was performed using the homologous structure of the Leishmania donovani chagasi FeSOD model (Mem. Inst. Oswaldo Cruz, suppl., 91:203, 1996) as a template, with which show high sequence identity. The model of the dimer thus obtained was of good quality as evaluated with respect to its stereochemistry and residue packing.

The active sites of the enzymes containing Fe or Mn, from several parasites, are very conserved, but in FeSOD from T. cruzi there is an interesting substitution of the W124, conserved in most FeSODs, by a leucine residue.

For both Leishmania and Trypanosoma, the development of these antiprotozoal drugs, which are chelating agents specifically designed to selectively disrupt the essential metal metabolism of T. cruzi, may lead to a new generation of pharmaceuticals for chemotherapeutic treatment of these diseases.

MULTIDRUG THERAPY IN EXPERIMENTAL LEISHMANIASIS IN BALB/C MICE

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There is no an efficient drug for treatment of Leishmaniasis. Many patients become resistant to treatment with antimonials which are the more utilized drugs and alternative treatment have been tried such as treatment with Interferon and with IL-2. Histologic Lesions of Cutaneous Leishmaniasis are very similar to those observed in Leprosy. In both it is seen two polar lesions. One with intense cellular hypersensitivity reaction and the other one with no cellular resistance. In Leprosy it has been used therapeutic scheme with association of two or more drugs. One of these drugs is Thalidomide. In the present study we used different schemes with one, or association of two or three drugs for treatment of Cutaneous Leishmaniasis. The drugs used were: Aminosidine - (2mg/mouse), Cyclophosphamide - (1mg/mouse), Thalidomide - (9mg/mouse) and Sodium Diclofenac - (5mg/mouse). 12 groups of 10 Balb/c were constituted for treatment and one group for control. Animals were infected with 1x10⁷ promastigote forms of L. amazonensis in the left foot pad. Treatment started 3 month after infection when the foot pad lesions rise 4 to 5 mm. Mice were sacrificed after 5 weeks of treatment. Treatment with Aminosidine gave excellent results when administered associated with Thalidomide and Sodium Diclofenac or associated with Thalidomide alone or Sodium Diclofenac reducing the lesions in 50% or more. Cyclophosphamide and Thalidomide did not have effect in the development of lesions having evolution similar to controls. Histopathologic study shows extensive areas of necrosis and less intense inflammatory process in the animals treated with Aminosidine alone or associated.

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MUTATION ANALYSIS OF CG2- CHLOROQUINE RESISTANCE RELATED GENE IN PLASMODIUM FALCIPARUM ISOLATES FROM THE BRAZILIAN AMAZON REGION - EVIDENCE OF CLONAL EXPANSION OF CHLOROQUINE RESISTANT PARASITE

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The first reports 40 years ago describing chloroquine resistance in *P. falciparum* pointed to an independent appearance in two foci. Strains appearing in Southeast Asia spreading to Africa on the one hand, and in South America on the other. Circumstantial evidence led to the belief that the overexpression or mutations of a P-glycoprotein (pfdmR1 gene) was the cause of chloroquine resistance. However, genetic cross analysis between resistant and sensitive strains did not show segregation of mutations in the pfdmR1 loci. A recently published paper demonstrates a strong correlation between a specific set of polymorphism in the cg2 gene of *P. falciparum* strains from Southeast Asia and Africa, and chloroquine resistance. The data on the only South American strain presented in this paper shows a different set of polymorphism. Analysis of 18 fresh cultured-stable *P. falciparum* isolates from the Brazilian Amazon region showed in vitro resistance to Chloroquine and Quinine. The cg2 repeats and mutation pattern were similar to the South American strain 7G8. This data could indicate that chloroquine resistance in the Brazilian Amazon is a clonal expansion of a single resistant parasite and could point to a different mechanism of resistance in South American strains.

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**CH-36**

**PANCREATITIS INDUCED BY SODIUM STIBOGLUCONATE (SBV) IN BALB/C TREATED MICE AFTER INFECTION WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS. COMPARISON WITH MEGLUMINE ANTIMONATE THERAPY**

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Sodium Stibogluconate (SbV) and Meglumine Antimonate (Glucantime) are the first choice drugs against all forms of Leishmaniasis. In Brazil, from 1996 to 1997 a Chinese formulation of SbV (BP88) was imported for the therapy of Leishmania infections in endemic areas without previous studies. The aim of the present work was to study the efficacy and toxicity of the Chinese ShV as compared to Glucantime, in the treatment of experimental infections of Balb/c mice with *L. amazonensis*. Thirty seven female Balb/c mice were infected in the left hind footpad with 5 x 10⁶ promastigotes of the MHOM/BR88/BA/125 strain and divided in 3 groups: G1: 18 mice treated with 100 ml (10mg) SbV/day; G2: 9 mice treated with 100 ml (~10mg) Glucantime/day; G3: 10 mice treated with 100 ml saline/day. Therapy was initiated 2 weeks after infection and injections were made by the intraperitoneal route for 21 consecutive days. Lesions size was monitored weekly using a dial caliper and expressed as the difference between the infected and contralateral footpad. The arithmetic means and standard errors were calculated and plotted against time of observation. All mice developed lesions at the site of inoculation regardless of the treatment applied. Only during the therapy was regression of the lesions observed. Analysis of the rate of lesion development showed a decreased variability in the ShV treated group. Nevertheless, mice treated with ShV were visibly more debilitated and had an episode of diarrhea when a second course of treatment was tried. Histopathological examinations of sections of heart, lungs, liver, kidneys, pancreas, spleen, intestines, popliteal lymphnode and infected footpad were made by H x E staining. Pancreatitis was observed in 17 of the 18 mice treated with ShV. The inflammation had a focal aspect but in most cases was disseminated throughout the organ. No pancreatitis was seen in mice of the Glucantime treated and control groups.

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**CH-37**

**PENTOXIFYLLINE AS AN IMMUNE MODULATOR DRUG ON THE EXPERIMENTAL CUTANEOUS LEISHMANIASIS (PRELIMINARY RESULTS)**

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In the animal model caused by *Leishmania (Leishmania) amazonensis* there are many lymphokines involved in the complex mechanism of the host-parasite reaction. In this present study we intended to interfere on the inflammatory reaction to the parasites, so that a better understanding of the pathogenic mechanisms of the lesion and to try to maximize its performance and/or to modify it, through immune modulation. Female isogenic mice were used, C5BL/6, some were inoculated on the right ear and some others on the right footpad with 3.10⁶ promastigotes on the stationary phase of the strain of MHOM/BR/PH8 of *Leishmania (Leishmania) amazonensis*. The group of animals treated since the immediate day of the inoculation received Pentoxifylline, 8 mg per Kg of mass, every 12 hours, through the period of 120 days and were sacrificed at the 40th, 80th and 120th day after the beginning of the treatment. Another group began the treatment with the drug 40 days after the inoculation, killed at the 80th and 120th day after the inoculation. A third group did not receive this treatment, so that it became the control group, they were also killed at the 40th, 80th, and 120th day after the infection. All the ears excised were analyzed in respect of the weight variation between both ears and in respect of the histopathologic analyses of the laminae. By the Limiting
Dilution method (Titus et al) a quantification of the parasites was done, on the footpads of the animals which were inoculated in this site. It was observed among the animals treated in relation to the control animals a significant reduction of the number of parasites, which had an accordingly significant reduction on the weight of ears of the treated animals. With respect to the histopathologic view, there was a considerable reduction of the participation of the vacuolated and parasite loaded macrophages, and of the level of global cellular infiltration. Finally, in the group treated after 40 days of infection, there was the observation of a reduction on the parasitism comparatively to the control group only on the 120th day after the inoculation. The Pentoxifylline, along with its already proved inhibition of the transcription of the mRNA for Tumour Necrosis Factor-alpha (TNF-α), modified the pattern of inflammatory response to the animal model under study. It reduced the macrophages propensity to vacuolation and yielded a greater effectiveness of these cells on the destruction of parasites. Besides, the group that began the treatment later demonstrated some not so expressive results in comparison to the earlier mentioned group. This fact may be explained by the role of the previously established infection.

This work was achieved with the aid of CNPq, FAPDF, UnB.

**CH-38**

PEPTIDES ISOLATED FROM SKIN SECRETION OF PHYLOMEDUSA TARSII ARE POTENT TRYPANOCIDAL AGENTS

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We denoted that skin secretion of *P. tarsius* is extremely toxic to *Trypanosoma cruzi*. Reverse phase HPLC on C18 Vydac column of the extract yielded 9 peaks, 3 of them are lytic in vitro to epimastigote, trypomastigote, and always to amastigote culture forms of *T. cruzi*. Materials of these peaks are poor lytic to normal BALB/c peritoneal macrophages. According to these results, we decided study the possible action of peptides in reducing parasitaemia and mortality ratio of *T. cruzi* infected mice.

Administration of peptides 5,6,7 and 8, diminished, from 2 to 1000 fold, the levels of parasitaemia in *T. cruzi* infected mice. Maximal parasitaemia was detected at day twenty three after infection. The average parasitaemia observed in groups of mice treated with total extract or saline at day 32 after infection were 879 and 1600 trypomastigotes/ml of blood, respectively. In contrast, the group of mice treated with peptides of peaks 5,6,7 and 8 showed only 240, 249, 53.3 and 17.7 parasites/ml of blood, respectively (p < 0.005). Mortality ratios of infected mice or mice treated with total extract, were 100%. The mortality ratio of infected mice given material of peak 5 was 66.6%; of peak 6 was 80%; of peak 7 was 44.4%; and of peak 8 was 22.2%, thirty days after infection (p < 0.001).

The administration of peptides 5,6,7 and 8, diminished, from 32 to 2000 fold, the levels of parasitaemia in *T. cruzi* infected mice of another group, and maximal parasitaemia was detected at day nine after infection. The average parasitaemia observed in groups of mice treated with total extract or saline at day 20 after infection were 569 and 568 trypomastigotes/ml of blood, respectively. In contrast, the group of mice treated with peptides of peaks 5,6,7 and 8 showed only 50, 160, 50 and 0.0 parasites/ml of blood, respectively (p < 0.05). Mortality ratios of infected mice or mice treated with total extract, were 90 to 100%. The mortality ratio of infected mice given material of peak 5 was 40%; of peak 6 was 66.6%; of peak 7 was 40%; and of peak 8 was 33.3% (p < 0.01).

**CH-39**

PRELIMINARY FORMULATION OF STRUCTURE-ACTIVITY RELATIONSHIPS FOR AMIDINE DERIVATIVES IN LEISHMANIA AMAZONENSIS PROMASTIGOTES AND AXENIC AMASTIGOTES

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In previous works, we reported the *in vitro* antileishmanial activity of a series of six amidine derivatives against *Leishmania amazonensis* promastigote (Canto-Cavalheiro, 1997). This work describes the *in vitro* antileishmanial activity of these derivatives in the axenic amastigote form of the parasite, the clinically relevant parasite stage. Possible structure-activity relationships were investigated using computational techniques.

The preliminary results showed significant *in vitro* anti-Leishmania activity of the derivatives tested in both, promastigotes and amastigotes forms of parasite, but not surprising, the susceptibilities of amastigotes and promastigotes to antileishmanial compounds tested are different and smaller for axenic amastigote. Parallely the reference antileishmanial agent pentamidine were less active for amastigotes than for promastigotes.

The theoretical calculations based on the semi-empirical AM1 method were performed with all compounds. The
most stable conformations were deduced from calculations and the resulting geometries were employed for the determination of theoretical electronic parameters like HOMO and LUMO energies, charges and dipole moments for each molecule. A analysis of the data and theoretical calculations showed that in vitro LD values correlated poorly with the HOMO energies of the studied compounds, casting doubt on an eventual involvement of charge transfer interactions between the receptor and the aromatic rings of compounds. On the other hand, this analysis indicate that the most relevant property calculate by AM1 in the activity is the dipole moment which correlates of a not linear form with the activity, however the physical meaning of this relation is not clearly and the contribution of other parameters, for example steric and lipophilic effect, must be considered for a detailed SAR analysis.

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CH-40
SCREENING PLANT EXTRACTS FOR TRYPANOCIDAL ACTIVITY AGAINST BLOOD TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI

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Nature is a very rich source of new compounds that can be developed into new drugs or research tools for almost all diseases. In this regard, we are evaluating plant extracts of the Brazilian flora for trypanocidal activity against trypomastigote forms of Trypanosoma cruzi present in murine blood obtained from experimentally infected mice. The plants parts were collected, powdered and extracted under vacuum in a Soxhlet apparatus with methylenechloride followed by methanol, or with mechanical stirring at room temperature with ethanol. The solvents were removed by evaporation under reduced pressure. Blood infected with trypomastigotes of T. cruzi Y strain was obtained by retro-orbital bleeding of experimentally infected male Swiss albino mice and diluted with normal murine blood to 2 x 10^6 trypomastigotes/mL. The plant extracts were assayed at a concentration of 500 µg/mL. Controls containing 2.5% DMSO or gentian violet at its IC50 (7.5 µg/mL) were run in parallel. After 24h at 4°C the number of parasites was determined by placing 5 µL of the tested blood on a glass plate, covering with a 22x22 mm coverslip, and counting the parasites in 50 fields at 400x magnification. Each experiment was performed in duplicate and repeated twice. The results were expressed as the percentage reduction of parasitaemia compared to the control with DMSO, that at 2.5% does not interfere with the parasite survival. A total of 212 plant extracts from 107 plant species were screened in this assay. Extracts from 5 different species eliminated more than 80% of the blood parasites, while extracts from 8 other plant species lysed nearly 50% of the parasites. In a previous work (Mem. Inst. Oswaldo Cruz, 92, Suppl.1, 1997), the in vitro activity of 42 plant extracts tested at 2.5 mg/mL against T. cruzi trypomastigotes obtained from tissue culture was described. From those, only 1 extract was active under the present assay conditions, reducing the parasite number in 86%. These results suggested either difference in drug susceptibility between trypomastigotes forms from culture and from blood or the effect of the different milieu the trypomastigotes are on during the drug screening. Our next step will involve a bioassay-guided chemical fractionation of the most active extracts in order to isolate and identify the active compounds and test them also in T. cruzi infected mice.

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CH-41
STUDIES ON THE EFFECT OF DINITROANILINE HERBICIDES IN TRYPANOSOMA CRUZI

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The dinitroaniline trifluralin (TF), (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzamine) is a microtubule-disrupting herbicide shown to be active against different pathogenic protozoa such as Cryptosporidium parvum (Arrowood et al., FEMS Microbiol. Let. 136:245,1996), Toxoplasma gondii (Stokkermans et al., Exp.Parasitol.84:355,1996), Plasmodium falciparum (Nath & Schneider, Clin. Res. 40:331A,1992, Kaidoh et al., J.Eukaryot. Microbiol. 42:61,1995), different species of Leishmania and T. brucei (Chen et al. Antimicrob.Agents Chemother. 37:1999,1993; Chen et al. PNAS 90:5657,1993) . Since this herbicide binds to plant but not animal tubulins, it is a promising lead compounds for antiparasitic agents. Drugs with microtubules as their targets have previously been used successfully in cancer therapy and antihelminthic drugs. It was claimed that the leishmanicidal activity was associated with chloralin (Cl) (4-chloro-3,5-dinitrobenzotrifluoride), a contaminant of TF synthesis (Callahan et al., Antimicrob.Agents Chemother. 40:947,1996). Preliminary work showed activity of TF against T.cruzi and an apparent relationship between susceptibility of TF and β-tubulin sequence of a given organism (Ortigão et al., Mem.Inst.Oswaldo Cruz 92 (suppl.I): 24, 1997).
In the present work it was analysed the effect of both compounds on the proliferation of *T. cruzi* epimastigotes (Y strain and clone DM28c) in LIT medium at 28°C. We observed significant differences between the behavior of the two drugs, in experiments with the Y strain, **Cl** was 100 times more active than **Tf** and comparing the two populations, the clone Dm28C about 8 times more susceptible than Y strain:

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<th>Trifluralin/Y strain</th>
<th>Trifluralin/clone m28c</th>
<th>Chloralin/Y strain</th>
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<td>ID50/3 days (mM)</td>
<td>677.0±55.8</td>
<td>82.9±0.2</td>
<td>6.0±0.3</td>
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</tbody>
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Ultrastructural analysis of parasites treated for 3 days with 100 µM **Tf** and 6 mM **Cl** caused damage to the mitochondria, with swelling of the organelle, and with the latter drug alterations on the kDNA network were also detected. Surprisingly, no characteristic damage to subpellicular microtubules of the parasite was observed by routine electron microscopy technique.

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**CH-42**

THE EFFECT OF METOXY-AMIDINE ON *LEISHMANIA AMAZONENSIS* IS ASSOCIATED TO THE TRYPANOThIONe REDUCTASE ACTIVITY


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The enzyme trypanothione reductase (TR), which is the parasitic homologue of glutathione reductase (GR) of mammals, appears to be one of the most promising targets for trypanocidal drugs. Trypanothione molecule, the natural substrate for trypanothione reductase, is a tripeptide associated to a poliamine, the spermidine. It has been demonstrated, that one of the proposed mechanism for the action of pentamidine on leishmania parasites seems to be related to the poliamine biosynthesis. In a previous work we described the efficiency of several amidines against *L. amazonensis* promastigotes, being the most effective the methoxy-derivative (Canto-Cavalheiro et al., 1997).

Based on our data, we decided to evaluate the effect of the methoxy-amidine on the activity of the trypanothione reductase of *L. amazonensis* promastigotes. The enzyme activity was followed up to 60 min in a mixture containing: 40mM HEPES, pH7.5, 1mM EDTA, 100 mM NADPH and the parasite sample (promastigote extract) in a concentration of 1mg/mL of protein. The reaction was measured in a spectrophotometer at 340nm, with relation to the consumption of NADPH. To assay the effect of the methoxy-amidine, in a concentration which killed 50 and 100% of the parasites, the same conditions were used besides the presence of specific (trypanothione) and non-specific (glutatione)substrates. The results showed that the highest activity occured on the 5th and 6th days, and that the parasite enzyme (trypanothione reductase), was able to use efficiently only the specific substrate with a high consumption of NADPH. In order to evaluate the drug sensitivity of the two evolutive stages of the parasite, *L. amazonensis* axenic amastigotes are being tested using the same protocol.

Supported by CNPq/Faperj/Fiocruz.

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**CH-43**

THE RELATIONSHIP BETWEEN IN VITRO ANTI-*TRYPANOSOMA CRUZI* ACTIVITY AND THE INTERMOLECULAR INTERACTIONS OF AROMATIC NITROGUANYL HYDRAZONES WITH BOVINE SERUM ALBUMIN AND DNA

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Aromatic guanyl hydrazones have been shown to be promising agents for the prophylaxis of blood tainted with *T. cruzi* and even for the chemotherapy of Chagas disease. The mechanism of action of these compounds is still unknown but it have been suggested that it may be through the interaction of the drugs with the DNA at the A-T rich regions of the minor grove, and more recently through an interaction of the drugs with the parasite membrane. The NMR relaxation studies and the molecular modeling of the solution behavior of the guanyl hydrazones of 2-nitrobenzaldehyde (2NBGH, ID50 87.5 µM), 3-nitrobenzaldehyde (3NBGH, ID50 182.6 µM) and 4-nitrobenzaldehyde (4NBGH, ID50 55.9 µM) showed that there is a direct relationship between the tendency of the drugs to form dimers with the values of ID50. The dimers of the most active of these isomers, 4NBGH, are 9.4 kcal/mol more unstable that the dimers of less active isomer, 3NBGH. The interaction of these drugs with BSA was studied measuring the selective and non selective longitudinal hydrogen relaxation times, T1S and T1NS of the drugs dissolved in 0.1 M
acetate buffer with and without BSA. The changes in $T_1^S$ and $T_1^{NS}$ observed were used to calculate the correlation times ($\tau_c$) of the drugs in the two environments. The results confirm the increasing tendency of the drugs to dimerization in the order $3NBGH>2NBGH>4NBGH$, and show that the interaction of the drugs with BSA is fast and reversible and that it follows the order $4NBGH>2NBGH>3NBGH$. The preliminary results of the studies with calf thymus DNA showed that, in the 0.1 M acetate buffer, there is not significant drug-DNA interaction.


CH-44
TREATMENT OF CANINE VISCERAL LEISHMANIASIS WITH GLUCANTIME® OR GLUCANTIMEPLUS IMMUNOTHERAPY

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Treatment of dogs visceral leishmaniasis is basically the same as the treatment of humans. However, cure is not usually achieved, leaving the sacrifice of the animal as the only feasible choice. The goal of this work was to test the therapeutic efficacy of N-methyl glucamine antimoniante (Glucantime®), alone or in association with immunotherapy, in dogs with asymptomatic visceral leishmaniasis. In this trial, 32 mongrel dogs, with ages ranging from 8 to 14 months, received inocula of $1 \times 10^7$ amastigotes of strain MHOM/BR/72/BH46 of *Leishmania chagasi*. They were monitored for 15 months. On a monthly basis, indirect immunofluorescence assay (IFA), enzyme linked immunosorbent assay (ELISA) and the rapid anti-Leishmania donovani antibody test (TRALd) were performed on blood samples of each animal. Bone-marrow aspirates were parasitologically investigated for *L. chagasi* by microscopic examination of Giemsa-stained smears and by cultivation in NNN/LIT culture medium. After confirmation of the infection, the animals were treated with subcutaneous inocula of a 1:10,000-merthiolated and sonicated extract of *L. braziliensis* (MCAN/BR/72/C348) promastigotes alone or in association with Glucantime®. Dogs were subdivided into four groups, each having eight animals: Group A, receiving 500mg/day of *L. braziliensis* antigen; group B, receiving 100mg/day Glucantime®; Group C, receiving, in association, the same dosage of Glucantime® and *Leishmania* antigen; and Group D, receiving no treatment. Both the antigen and Glucantime® were administered subcutaneously during 20 consecutive days, following a 10-day interval, second series of injections, second 10-day interval and final 20-day long treatment. Although statistically significant differences among Groups A/C, B/D and C/D as evaluated by the IFA test, detecting a decrease in the antibody levels 210 days after the conclusion of the treatment, no negativation of the tests was observed. Within the same time period, the ELISA test showed no difference between Groups C and D. The TRALd test was ever positive. At 450 days post-infection, dogs were sacrificed. *L. chagasi* was searched for histologically in biopsies of the bone marrow, lymph nodes from the popliteal and mesenteric regions, spleen, liver, skin fragments from the nasal region and tips of the ears. Thirteen animals were found parasitologically positive: 4 from Group A, 1 from Group B, 3 from Group C and 5 from Group D. Our conclusion is that the treatments of groups B and C were effective in reducing the antibody levels and parasitism of the dogs, but failed to promote cure.

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CH-45
TREATMENT OF EXPERIMENTAL CHAGAS’ DISEASE USING SEVERAL DRUGS AGAINST *TRYPAÑOSOMA CRUZI*, ASSOCIATED TO HORMONAL AND NON-HORMONAL ANTI-INFLAMMATORY

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The aim of this study was to, at experimental level, for an effective treatment against *T. cruzi* using a drug collection with some efficiency over Chagas’ disease associated with hormonal and non-hormonal anti-inflammatory drugs (benznidazol, levamizol, allopurinol, verapamil, indometacin and dexametasona).

Non isogenic male mice used and divided into seven experimental groups. Six groups were infected with Vicentina *T. cruzi* strain and one was the negative control group: G1: benznidazol, G2: levamizol, G3: anti-inflammatory (indometacin or dexametasona), G4: allopurinol or verapamil, G5: association of 4 drugs (benznidazol + levamizol + allopurinol or verapamil + dexametasona or indometacin), G6: positive control group, G7: negative control group.
When 50% of the animals showed positive parasitaemia, the treatment started. The drugs were administered per os for 20 consecutive days. The parasitaemia and mortality were examined daily, 30 days.

Benznidazol showed to be effective on reducing parasitaemia and controlling mortality, but the results were dependent on the dose; allopurinol and verapamil did not present suppression effect on T. cruzi; levamizol delayed parasitaemia peak but presents high levels of blood parasites and mortality; anti-inflammatory drugs induced the higher parasitaemia and mortality in all the experiments. When the association between all the drugs and anti-inflammatory (indometacin or dexametasona) was used, it was observed that the group that received indometacin showed reduction of parasitaemia but high mortality; when the mice received the association of drugs plus dexametasona occurred decrease in parasitaemia and mortality. Amazingly, when the association (benzimidazol, levamizol, verapamil and indometacin) was used the highest protection was observed.

In conclusion, this paper suggest that other associations with drugs aiming a possible sinergic effect should be carried out.

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**CH-46**

**TREATMENT OF EXPERIMENTAL CUTANEOUS LEISHMANIASIS WITH TOPICAL FORMULATIONS OF PAROMOMYCIN**

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Paromomycin (PA), an aminoglycoside antibiotic, has been tested in an ointment as an alternative treatment against cutaneous leishmaniasis, with a high efficacy at the concentration of 15%. Although this treatment has shown promising results it has not been successful in accelerating the recovery of most cases. This could be attributed to the low penetration of PA into skin. To investigate this hypothesis three different formulations containing 5% PA were prepared (ointment, oil(o)/water(w) and w/o/w emulsions). Control preparation was made in w/o/w emulsion without PA. These formulations showed in vitro enhanced PA penetration into skin. We compared the efficacy of them by treating local dermal lesions of L. major infected BALB/c mice. Four groups of ten mice were inoculated at the base of the tail with 5 x 10⁶ promastigotes and after lesion development they were treated twice daily for a period of twelve days. Lesion development was followed macroscopically by measuring lesion size. Biopsy material was taken for histological analysis. Quantification of parasites in infected tissues was performed by limiting dilution assays and levels of IgG2a and IgG1, which correlate with Th1 and Th2 responses respectively, were measured by ELISA. Ten days post treatment, mice treated with o/w emulsion and ointment presented an average reduction of 60% in lesion size in comparison to control and w/o/w emulsion treated mice. A chronic and productive inflammation with microabscess formation, necrosis and an exsudate composed by lymphocytes and macrophages was observed in all groups. Heavily infected macrophages with L. major amastigotes were also present. However, in the group treated with ointment and also in one animal of the group treated with o/w emulsion was observed more intense fibroblastic proliferation and collagen deposition. Moreover, lesions were completely resolved in two animals of the group treated with o/w emulsion and in three of the one treated with ointment. A correlation among lesion resolution, absence of inflammation, lower number of parasites and a discrete enhancement of the IgG2a level was observed in one animal of each of these groups. Although our in vitro studies showed that o/w emulsions would be the best preparation for topical delivery of PA, no difference was observed in vivo between this preparation and the topical application of ointment. Our results are in agreement with the drug activity described by others using the same concentration of PA in ointment. Other studies are necessary to improve the efficacy of this preparation.

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**CH-47**

**TRYPANOCIDAL ACTIVITY AND CHEMOKINES MRNA EXPRESSION BY CULTURED MICE MYOCARDIAL CELLS INFECTED WITH TRYPANOSOMA CRUZI**

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The progressive cardiac tissue damage observed during the infection with T. cruzi is thought to be due to autoimmune phenomena, but response to parasites persisting in the heart cannot be ruled out.

To understand the pathogenesis of myocarditis, it is important to know the mediators that trigger leukocyte migration to the heart. We have previously shown that mRNA for different chemokines are produced in the heart of mice acutely infected with T. cruzi. Also, this mRNA expression correlates with the type and intensity of the inflammatory infiltrated. The heart tissue also expressed mRNA for the inducible nitric oxide synthase (iNOS). In this work we evaluated the role of myocardial cells in the production of chemokines, cytokines and in the activation
of iNOS. Normal murine embryonic myocardial cells were cultured with live trypomastigote forms of *T. cruzi* and mRNA expression was assessed by RT-PCR method. Since NO has been implicated as very important mediator of parasite killing, we evaluated if cardiac cells exhibit trypanocidal activity following stimulus with different cytokines. We found that trypomastigote forms of *T. cruzi* induced iNOS mRNA expression and low levels of nitrite were detected in the cultures. The addition of IL-1β, IFN-γ or TNF-α to the myocardial cell cultures resulted in significant NO production, although the cells exhibited only low trypanocidal activity. However, high levels of NO and a marked trypanocidal activity were observed when IL-1β, IFN-γ and TNF-α were simultaneously added to the cultures. Trypanocidal activity was mediated by iNOS/L-arginine pathway, since it was inhibited by treatment with L-NMMA. In addition, myocardial cells infected with *T. cruzi* expressed mRNA for the chemokines KC, JE, Crg-2, RANTES, Mig and to the cytokine TNF-alpha. In summary, our results suggest that myocardial cells mediate NO-induced trypanocidal activity and play a role in the modulation of cell recruitment. These results indicate that pro-inflammatory cytokines and chemokines produced within the myocardium are likely to control the parasite growth, cell influx and to participate in the pathogenesis of acute chagasic cardiomyopathy.

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CH-48

TRYPANOCIDAL ACTIVITY OF HYDROXYLATED DERIVATIVE OF N,N-DIMETHYL-2-PROPEN-1-AMINES

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The 3-(4´-bromo-[1,1´-biphenyl]-4-yl)-3-(4-X-phenyl)-N,N-dimethyl-2-propen-1-amine compounds have been shown to be active against *Trypanosoma cruzi* (De Conti et al., Microbios 85:83, 1996; Pereira et al., Acta tropica 69: 205, 1998). Studies on chemical structure-biological activity relationships (SAR) were investigated using computational techniques. Theoretical calculations based on the semi-empirical AM1 method were performed with all compounds. Parameters like HOMO and LUMO energies, molecular volume and dipole moments are important for the molecule activity. In order to prove the SAR studies new compounds were synthesized with OH group at the para position of the phenyl ring (X=OH). The 3-(4´-bromo-[1,1´-biphenyl]-4-yl)-3-(4-hydroxylphenyl)-N,N-dimethyl-2-propen-1-amine (I) and 3-([1,1´-biphenyl]-4-yl)-3-(4-Hydroxylphenyl)-N,N-dimethyl-2-propen-1-amine (II) were synthesized. Both derivatives were characterized by infrared, 1H-Nuclear Magnetic Resonance (NMR) and Mass Spectrometry. The trypanocidal activity was assayed against bloodstream trypomastigote forms of *T. cruzi* (Y strain) through the parameter ID 50 /24h/4°C. The ID 50 values were 29.1 ± 2.1 μM for (I) and 25.7 ± 1.7 μM for (II). These results showed that the bromo at the para position of the biphenyl ring seems not to be important to trypanocidal activity of this molecule. Also, the trypanocidal activity of (I) showed that the SAR previous studies can predict a more active compound which could be synthesized in the next stage.

Support: Fapesp, Fiocruz

CH-49

TRYPANOXYDAL EFFECT OF MULIN-11,13-DIEN-20 OICO ACID IN MICE INFECTED WITH TULAHUEN STRAIN OF *TRYPANOSOMA CRUZI*

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Chemotherapy of Chagas’ disease is still an unresolved problem. Treatment is only possible in the acute infection, few drug are available and side effect have been observed. For these reason research and discovery of new compounds with trypanocidal activity are necessary.

We have previously demonstrated that mulin-11,13-dien-20 oico acid, a new diterpenoid isolated from *Mulinum crassifolium*, has an *in vitro* activity against 3 stages of *Trypanosoma cruzi*. The proposal of this communication is evaluate the *in vivo* trypanocidal effect of mulin-11,13-dien-20 oico acid. C3H mice were infected with 1 x 10⁶ trypomastigotes (Tulahuen strain). After that, 3 different groups of mice were injected with a single dose of mulin-11,13-dien-20 oico acid (20, 40 or 80 mg/kg/weight). Each mouse was bleed after 48 h and parasitemia curves and haematological aspect were studied.

Mulin-11,13-dien-20 oico acid was trypanocidal at concentration as low as 20 mg/kg. This compound was apparently atoxic for mice because haematologic parameter as number and morphology of erythrocyte, leukocyte and platelets were not affected.

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CH-50
VERAPAMIL INHIBITS THE ENERGY-DEPENDENT EFFLUX OF A DOXORUBICIN DERIVATIVE IN LEISHMANIA PROMASTIGOTES

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We previously identified, in intact Leishmania promastigotes, energy-dependent efflux pump(s), through its ability to transport three substrates of the mammalian multidrug resistance (MDR) transporters: calcein (CAL), calcein acetoxy-methyl ester (CAL-AM) and the doxorubicin derivative, pirarubicin (PIR). Furthermore, we showed that phenothiazines, which inhibit the activity of the mammalian MDR transporters, were also inhibitors of the Leishmania efflux pump.

In the present work, we evaluated the ability of verapamil, an other well-known MDR reversing-agent, to inhibit the efflux of CAL, CAL-AM and PIR in three different strains of Leishmania promastigote (L. guyanensis, L. braziliensis and L. mexicana). We found that verapamil inhibits, in a dose dependent manner, the efflux of PIR in the three Leishmania strains. However, no effect of verapamil on the effluxes of CAL and CAL-AM was observed (the highest concentration tested was 20 microM).

It is the first time that an effect of verapamil on a biochemical function is reported in Leishmania. That may be the mechanism by which verapamil reversed the resistance of Leishmania to antimonials (Sb(V)). In this context, the data presented here also suggests that Sb(V) resistance could be mediated by the PIR active extrusion system.

This work was supported by Université Paris Nord (France), CNPq, Fapemig, Pronex (Brazil).

CH-51
ULTRASTRUCTURAL ALTERATIONS ON LEISHMANIA AMAZONENSIS TREATED WITH PESCHIERA AUSTRALIS CHLOROFORM FRACTION

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Leishmaniasis is still a major world health problem, with around 3 million people infected and 600 thousand new cases appearing each year. Pentavalent antimony compounds are still the first line treatment for leishmaniasis but, disadvantages such as costs, long-term treatment and side effects are reported. Also, unresponsiveness has become a serious clinical problem. All this, prompted the search for new chemotherapeutic agents. Peschiera australis is an arbustive plant, popularly known in Brazil, that we previous investigated the anti-leishmanial activity in stem crude extract using a bioassay-guided fractionation. We showed anti-leishmanial activity in the chloroform fraction both, for promastogotes and amastigotes forms. The effect on amastigote was assessed on L. amazonensis infected moused peritoneal macrophages determining the parasite survival after 72hr, following two different protocols: a) only one treatment 1hr after the infection (1X) and b) addition of the chloroform fraction once a day during three days after infection (3X) Glucantime® was used for comparison following the same protocols above. A decrease in the parasite survival of 90% was observed after only one treatment with 10µg/mL of the chloroform fraction, while Glucantime®, showed a decrease of 60% at the same concentration and protocol. The IC50 of the chloroform fraction in this experiment was 2.6µg/mL and the IC50 of Glucantime® was 18µg/mL. No significant alteration in morphology, spreading and adherence of macrophages cultured in the presence of the chloroform fraction was observed at light microscopy. Transmission electron microscopy was done to better analyse the treatment. Infected and non-infected mouse peritoneal macrophages were treated 3X with 20 mg/mL of the chloroform fraction and 1% DMSO as a solvent control. The non-infected macrophage showed an increase in the cytoplasmic vacuoles when treated with the chloroform fraction, however the general structure of the cell, mainly mitochondria (the organelle affected by this drug in promastigotes) are intact and similar to non-treated controls. In the same manner, 1% DMSO did not change the macrophage ultrastructure. Viable amastigotes were not detected in the parasitophorous vacuole of macrophages treated 3X with 20 mg/mL of the chloroform fraction.

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CH-52
PYRUVATE PHOSPHATE DIKINASE: A NOVEL POTENTIAL TARGET AGAINST TRYPANOSOMA CRUZI

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We have cloned and characterized a gene that encodes a putative pyruvate phosphate dikinase (PPDK) from Trypanosoma cruzi. PPDK catalyses the reversible conversion of phosphoenolpyruvate to pyruvate in energy metabolism. This enzyme is absent in mammalian cells, but has been found in a wide variety of other organisms. In certain parasitic protists and some eubacteria it has a glycolytic function in the conversion of phosphoenolpyruvate, AMP and pyrophosphate to pyruvate, ATP and inorganic phosphate. In photosynthetic bacteria and in plants PPDK operates mainly in the gluconeogenic direction (i.e. conversion of pyruvate to phosphoenolpyruvate). Recently, PPDK has been found in Trypanosoma brucei, where it is present as a tandem array of two copies (PPDK1 and PPDK2), localized on a 1 Mbp chromosome. Northern and Western blot analyses showed that PPDK is transcribed and translated in epimastigote and trypomastigote forms as a protein of 100 kDa. The deduced protein sequence of PPDK1 indicates a protein of 100.8 kDa with a C-terminal AKL sequence, a signal for glycosomal import. Preliminary kinetic studies in crude extracts of epimastigotes, assayed in the reverse direction (i.e. formation of PEP from pyruvate, ATP and P_i), confirmed the presence of this enzyme activity. The expression and enzymatic characterization of the recombinant protein is under way. The functional role of this enzyme in Trypanosoma cruzi remains to be determined.

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CH-53
BENZnidazole TREATMENT OF INFECTED MICE ENHANCES PHAGOCYTOSIS AND CYTOKINE RELEASE BY MACROPHAGES DURING INFECTION WITH A DRUG SUSCEPTIBLE TRYPANOSOMA CRUZI STRAIN BUT NOT WITH A DERIVED DRUG-RESISTANT POPULATION

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To further investigate the cooperative effect of benznidazole (BZ) treatment and macrophage activation during therapy of acute phase of Chagas’ disease, we used populations and clones of T. cruzi, Y strain wild-type and resistant to BZ, previously selected in vivo (Murta & Romanha, Parasitology 1998, 116: 165-171). Mice infected with either parasite populations were treated at the peak of parasitemia with a single high dose of 500mg of BZ/kg of body weight. The trypomastigotes were isolated from infected mice 3h after treatment with BZ and not treated. The recovered parasites were incubated with inflammatory macrophages and used to study phagocytosis, cytokine release and reactive nitrogen intermediates (RNI) synthesis. Phagocytosis and destruction of the wild-type parasites were significantly enhanced after drug treatment. These enhancements were accompanied by increasing of the cytokines IL-12(p40) and TNFα and RNI release by murine inflammatory macrophages primed with INFγ. In contrast, BZ treatment of drug-resistant T. cruzi population had no effect on phagocytosis, cytokines release or RNI synthesis. Considering the importance of IL-12 and TNF-α on induction of IFN-γ and RNI, respectively, we measured the synthesis of these molecules by splenocytes of animals infected with either wild-type and drug-resistant parasite populations, before and 5h after treatment with BZ. The results in vivo are in agreement with those in vitro. Thus, after treatment with BZ, splenocytes from animals infected with the drug-susceptible Y strain of T. cruzi produced higher levels of INF-γ and RNI. Our findings indicate that BZ acts in the drug-susceptible T. cruzi strain enhancing the phagocytosis and the production of cytokines and RNI, thus, favoring the destruction of the intracellular parasites by the cellular compartment of the immune system. This early event does not occur in the resistant population infected mice treated with BZ and could be a key element in the understanding of the low efficacy of treatment in Chagas’ disease.

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