CH-1
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

Alwen A, Deps P, Portal AC, Oliveira AP, Araujo MV, D Reynaldo
USA Medical Research Unit and Núcleo de Doenças Infecciosas, Centro Biomédico, UFES, Vitória, ES, Brasil

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 brasiliensis* 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.

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

Torres MA, Echevarria A*, Canto-Cavalheiro MM, Cysne-Finkelstein L**, Alves LV, Fonseca L, Leon LL
Departamento de Imunologia, Instituto Oswaldo Cruz *Departamento de Química, Universidade Federal Rural de Rio de Janeiro - UFRRJ, Rio de Janeiro, RJ, Brasil **Departamento de Protozoologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

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 promissing 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. Parallelly, the reference antileishmanial agent pentamidine were less active for amastigotes than for promastigotes.

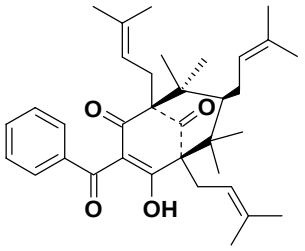
Theoretical calculations based on the semi-empirical AM1 method were performed with all compounds and theoretical electronic parameters like HOMO and LUMO energies, charges and dipole moments for each molecule were obtained. In general, the electronic factors, are responsible for alterations of the physical 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.

Supported by CNPq, Faperj, Fiocruz

CH-3
ACTIVITY OF 7-EPICLUSIANONE AGAINST BLOOD TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI*

Alves TMA, Alves RO*, Santos MH**, Nagem TJ***, Romanha AJ*, Zani CL
Laboratório de Química de Produtos Naturais *Laboratório de Parasitologia Celular e Molecular, CPqRR, Fiocruz, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brasil **Departamento de Química, ICEX, UFMG, Av. Antônio Carlos 6627, 31970-971 Belo Horizonte, MG, Brasil ***Departamento de Química, UFOP, 35400-000 Ouro Preto, MG, Brasil

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 4^0C 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.*



7-Epiclusianona

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.

Drug	Concentration (mg/mL)	Trypanocidal Activity (% ± S.D.) [a]
7-epiclusianone	500	92±1.0
	250	38±3.7
	125	17±1.2
Controls		
Without drug	-	0
Gentian violet	7.5	47±1.7
DMSO	2.5%	2±4.8

[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

Croft SL, Smith A, Rock P, Yardley V, Rhodes J*
Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK, *Glaxo Wellcome Research and Development, Medicines Research Centre, Stevenage, Herts SG1 2NY, UK

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-g, 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-g 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 1mM. 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)

CH-5
ANTIPARASITIC ACTIVITY AGAINST DIFFERENT STAGES OF *TRYPANOSOMA CRUZI* OF BIOMOLECULES ISOLED FROM *AZORELLA COMPACTA (UMBELLIFERAE)*

Araya JE, Neira I, Da Silva S**, Mortara R**, Manque P**, Porcile P, Sagua H, Loyola A*, Morales G*, Bórquez J*
Unidad de Parasitología, Facultad Ciencias de la Salud,Universidad de Antofagasta, Chile * Laboratorio Química de Productos Naturales, Facultad Ciencias Básicas, Universidad de Antofagasta, Chile ** Departamento de Micro-Imuno-Parasitologia, EPM,Unifesp, São Paulo, Brasil

Trypanosoma cruzi is the aethiological agent of Chagas'disease, an illness of considerable morbidity and mortality in Latinamerica. 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 a therefore an urgent need. In this study we are evaluated by bioassay-guided protocol the trypanocidal activity of molecules isolated from *Azorella compacta, Umbelliferae* family, an authoctonous medicinal plant of Andes Mountains of Norththern of Chile. The trypanocidal activity of diterpenoide 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 (DL_{50}) 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 grow was inhibited ~60 % in different strains assayed, with a DL_{50} 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.

CH-6
ANTIPROLIFERATIVE EFFECTS OF AJOENE IN PROMASTIGOTES AND AMASTIGOTES-LIKE FORMS ON SEVERAL *LEISHMANIA* SPECIES

Ledezma E, O'Daly JA*, Jorquera A, De Sousa L, Apìtz-Castro R*
Centro de Investigaciones en Ciencias de la Salud, Escuela de Medicina, Universidad de Oriente, Núcleo de Anzoátegui, AP 4774, Puerto la Cruz 6023, Estado Anzoátegui, Venezuela *Instituto Venezolano de Investigaciones Cientificas (IVIC) Venezuela

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 patogens. 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.

CH-7
ANTI-TRYPANOSOMATID TARGETS: GLYCOLYSIS AND PROTEIN PRENYLATION

Aronov A,Yokoyama K, Buckner F*, Suresh S**, Bressi J, Choe J***, Stuart K***, Van Voorhis W*, Verlinde C**, Hol W****, Gelb M****
Departments of Chemistry *Medicine **Biological Structure ***Pathobiology ****Biochemistry, University of Washington, Seattle, WA 98195 USA

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 K_I 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 anti-cancer agents, one may be able to extend the medicinal chemical and clinical pharmacological studies currently in progress to the development of anti- trypanosomatid agents.

Supported by National Institutes of Health and the Howard Hughes Medical Center

CH-8
BIOCHEMICAL CHARACTERIZATION OF THE RESISTANCE DEVELOPMENT OF *LEISHMANIA MEXICANA* TO GLIBENCLAMIDE

Mendoza-León A, García N*, Ponte-Sucre AI*
Laboratory of Biochemical and Molecular Biology of Parasites *Laboratory of Molecular Physiology, I.M.E., Universidad Central de Venezuela, Apartado 68256, Caracas 1062-A Fax: 58.2.693.4351, e-mail: aiponte@reacciun.ve , amendoza@strics.ciens.ucv.ve.

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 emphazises the need for the comprehension of the biochemical and molecular mecanisms 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 flagelar 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. terentolae* 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.

Supported by the Venezuelan grants FAC.MED 007.95, CDCH-UCV, 03.10.4126.98, 09.33.103515.95, 09.33.3514.95 09.33.4045.97.CONICIT RPIV-110034, S1-96001411

CH-9
CHEMOTHERAPEUTIC EVALUATION OF IMIDAZOLIC DERIVATES ON EXPERIMENTAL CHAGAS' DISEASE

Oliveira Cardoso C, Santos Silva A, Bittar PCM, Lages-Silva E, Ramírez LE
Departamento Ciências Biológicas, FMTM, Uberaba, MG, Brasil

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, meanwile 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 ketonocazole association and benznidazole, respectively, presented higher efficient on the reduction of parasitaemia, but not totally. The other drugs were ineffective.

Supported by Fapemig, Funepu.

CH-10
CHLORAMBUCIL: AN ALTERNATIVE DRUG TO ACUTE CHAGAS DISEASE TREATMENT

Silva SF, Santos W, Rodrigues AM, Carvalhaes MS
Laboratório de Imunoquímica, IPTSP, Universidade Federal de Goiás, Goiânia, GO, CP 131, Brasil

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 alkylanting 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^{-3}M, 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^{-4}M 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*

Gomes-Cardoso L, Echevarria A*, Aguiar-Alves F, Jansen AM**, Leon LL
Departamento de Imunologia, Instituto Oswaldo Cruz *Departamento de Química, Instituto de Ciências Exatas, UFRRJ **Departamento de Protozoologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

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 160mg/mL to 5mg/mL. In the present study the parasites were isolated from a dog and a coati, maintained in immunossupressed 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 (LDs50=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 (LDs50=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.

Supported by CNPq, Faperj, Fiocruz.

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

Jorge AS, Silveira TGV, Lonardoni MVC, Arraes SMAA, Zanzarini PD, Silva CM, Mello JCP, Bertolini DA
Departamento de Análises Clinicas e Departamento de Farmácia e Farmacologia, Universidade Estadual de Maringá, Av. Colombo 5790, 87020-900 Maringá, PR, Brasil

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 25ºC 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* for the promastigote forms of *L. (V.) braziliensis* and decreased the lesions *in vivo.*

Supported by CNPq, UEM

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

Chiuchetta SJR, Pupulin ART
Parasitologia e Bioquímica Básica, Universidade Estadual de Maringá, CP 331, 87020-900 Maringá, PR, Brasil

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 Benzonidazole on the mitochondrial oxidative metabolism of the same strains. For *in vivo* study, groups of 30 mice were used which were inoculated with $1x10^7$ 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 $3x10^8$ 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 ressuspended 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 reveled inhibited basal respiration of the mitochondrial in relation to succinate and glutamate.

Supported by FNS, Pibic, CNPq .

CH-14
EFFECT OF TERPENES IN CULTURES OF *PLASMODIUM FALCIPARUM*

Planchart RA, Machado MR, Stroeder H, Lopes NP*, Kimura EA, Peres VJ, Katzin AM
Departamento de Parasitologia, ICB, USP, 05508-900 São Paulo, SP, Brasil *Departamento de Química e Física FCFRP, USP, 14040-903 São Paulo, SP, Brasil

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_{50}/48 hours compared with mefloquine, which is currently used in malaria therapeutics. Values of IC_{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_{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

CH-15
EFFECT OF TREATMENT WITH TRYPANOCIDAL NITRODERIVATIVES IN THE PROGNOSIS OF CHAGAS' DISEASE PATIENTS NOT SUBJECTED TO EXOGENOUS SUPERINFECTIONS

Lauria-Pires L., Braga ML, Alves NP, Argañaraz ER, Simões-Barbosa AM, Tinoco DL, Teixeira ARL
Laboratorio de Pesquisa Multidisciplinar em Doença de Chagas, Universidade de Brasília, DF, Brasil

We have provided medical care to the streat-cleaners of Brasília, Federal District. Among 718 streat-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
EFFECTIVITY OF TRICYCLIC DRUGS FOR *TRYPANOSOMA CRUZI* INFECTION TREATMENTS MEASURED BY? CARDIAC RECEPTORS FUNCTION

Fernández AR, Enders JE, Rivarola HW, Fretes R, Palma JÁ, Paglini P
Cát. Física Biomédica, Cát. Histología, Fac. Cs. Médicas (UNCba), Santa Rosa 1085, 5000 Córdoba, Argentina

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 effectivity of some tricyclic drugs used in clinic(Thioridazine (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 analized. ?-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 ß 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

Almeida RP, D'Oliveira Jr A, Machado PR, Ko A*, Mobasherry N*, Ribeiro de Jesus A, Bacellar O, Carvalho EM
Serviço de Imunologia, HUPES, UFBA, Salvador, BA, Brasil *Cornell Univ. Med. College, NY, NY, USA

Pentavalent antimonials are standard treatment of Cutaneous Leishamniasis (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 (20mg/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 $\pm$ SD of healing time of the rGM-CSF group 49$\pm$38 days and control group was 110$\pm$62 ($p=0.02$). GM-CSF increased the levels of IFN-gfrom 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 abillity of GM-CSF to induce scar formation.

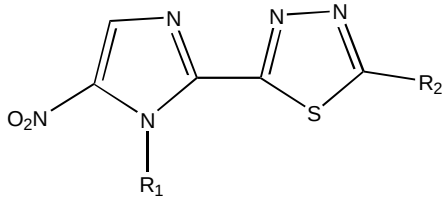
Supported by Finep, CADCT, Pronex.

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

Nogueira CM, Silva EF, Lima ELS, Barbosa ACM, Fernandes RC, Fernandez-Ferreira E, Soares ROA, Gibaldi D, Ribeiro dos Santos R
Farmacologia Aplicada, Far-Manguinhos, Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

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 benzinidazole (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].



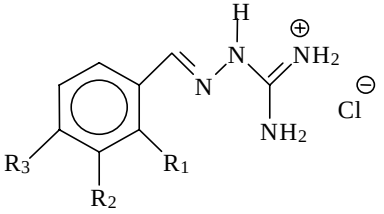
Megazol: R_1= CH_3, R_2 = NH_2; Mega-A: R_1= CH_3, R_2 = $NHCOC_3H_6CO_2H$; Mega-T: R_1= CH_3, R_2 = SCN_2H_3; Mega-G: R_1= CH_3, R_2 = OCH_2CH_3; Mega-S: R_1= $CH_2CH_2SO_2CH_2CH_3$, R_2 = NH_2

Different of 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

Messeder JC, Figueroa-Villar JD, Molina J*, Brener Z*
Departamento de Química, Instituto Militar de Engenharia, Praça General Tibúrcio 80, 20290-270 Rio de Janeiro, RJ, Brasil (messeder@aquarius.ime.eb.br) *Laboratório de Doença de Chagas do Centro de Pesquisas René Rachou Fiocruz, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brasil

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 ^{13}C nuclear magnetic ressonance (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.



R1	R2	R3	ID_{50}(mg/mL)
Cl	H	H	0,30
H	Br	H	0,25
H	H	Br	0,25
H	H	OCH_3	0,50
H	OCH_3	OCH_3	0,30

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 substitutes 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

Levy AMA, Caovilla FCM, Fragata Filho AA, Lourenço AM, Pereira-Chioccola VL, Ramos MC
Instituto Dante Pazzanese de Cardiologia, Av. Dante Pazzanese 500, 04012-180 São Paulo, SP, Brasil

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 therapeutical 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*

Santa Rita RM, Barbosa HS, Meirelles MNL, Bisaggio RC*, De Castro SL
Departamento de Ultra-estrutura e Biologia Celular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Lab. Imunobiofísica, IBCCF, UFRJ, RJ, Brasil

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} (mM) are displayed below:

	epimastigotes				amamastigotes			
h	***24***	***48***	***72***	***96***	***24***	***48***	***72***	***96***
Ed	11.4±0.2	6.5±1.3	5.1±0.3	5.0±0.4	17.8±2.2	4.2±1.6	3.5±0.9	2.3±0.5
Mit	26.6±3.7	9.3±0.3	6.4±0.8	3.4±1.1	18.6±6.8	7.9±1.9	7.4±2.5	4.8±0.4
Ilm	17.4±1.2	9.2±0.0	8.0±0.0	7.7±0.0	11.6±0.1	4.5±0.1	4.2±0.1	2.5±0.6

To measure intracellular calcium levels the parasites were loaded with fura-2, resuspended in PBS, and the ALPs (2.5 to 15 mM) was added directly into the cuvette. The effect of the drugs on *T.cruzi* was more more pronouced 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 characteristically, alterations on the flagellum membrane with strong vesiculation.

The pre-treatment of trypomastigotes for 1h a 37°C with 30 mM Ed followed by washings and ressuspension 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. Mantaining 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 mM.

Supported by EC (INCO-DC IC18-CT96-0084), CNPq, Papes, Fiocruz.

CH-22
GROWTH INHIBITION AND ULTRASTRUCTURAL CHANGES INDUCED BY LICHENIC SUBSTANCES ON *LEISHMANIA CHAGASI IN VITRO*

Jota VC, Silva NH, Pereira EEC**, Figueiredo RCB***, Andrade PP*
Departamento de Bioquímica *Departamento de Genética **Departamento de Botânica, Universidade Federal de Pernambuco, Av. Moraes Rego s/n, Recife, 50670-901 PE, Brasil ***Centro de Pesquisas Aggeu Magalhães, Fiocruz, Recife, Pernambuco, Brasil

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 ^oC, 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-containg 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 lichenic substances as potential new drugs in leishmaniasis.

CH-23
GROWTH INHIBITION OF *LEISHMANIA (LEISHMANIA) AMAZONENSIS* BY AUSTRALIAN SNAKE VENOMS

Lourenço CO, Tempone AG, Spencer PJ, Andrade Jr HF*, Mirtschin PJ**, Nascimento N
Divisão de Radiobiologia, IPEN, CNEN, SP, Brasil *Protozoologia, L. IMTSP, FMUSP, SP, Brasil **Venom Supplies, South Australia

Cutaneous leishmaniasis is an endemic disease, mostly found in tropical and subtropical regions. In Brazil, it is registrated 26000 new cases/year considering all their clinical forms. The actual treatment is based on some toxic drugs such as pentamidine and antimonials. 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 40µg/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^OC. 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.

CH-24
IMMUNESUPPRESSION REDUCES THE ACTIVITY OF STEROL BIOSYNTHESIS INHIBITORS (SCH56592 AND DO870) BUT NOT BENZNIDAZOLE (BZ) IN MICE ACUTELY INFECTEDWITH *TRYPANOSOMA CRUZI*

Molina JT, Urbina JÁ**, Martins-Filho OA, Romanha AJ*, Brener Z
Lab. Doença de Chagas Lab. Parasitologia Celular e Molecular do Centro de Pesquisas René Rachou, Fiocruz, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brasil ** Lab. Química Biológica, CBB, IVIC, Apartado 21827, Caracas, 1020AVenezuela.

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 immunecompetent mice. SCH56592 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

Castillo V, Neira I*, Olivares A**, Sagua H.*, Bórquez J***, Loyola A***
Unidad de Hematología *Unidad de Parasitología **Depto. de Acuicultura ***Grupo de Química, Productos Naturales, Universidad de Antofagasta, Antofagasta, Chile

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 (LD_{50}) 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

Murta SMF/***, Martins-Filho OA*, Corrêa-Oliveira R**, Romanha AJ***
Departamento de Bioquímica e Imunologia, ICB, UFMG, Caixa Postal 486, BH, MG, *Lab. Doença de Chagas, **Lab. Imunologia Celular e Molecular ***Lab. Parasitologia Celular e Molecular, Centro de Pesquisas René Rachou, Fiocruz, Caixa Postal 1743, 30190-002 Belo Horizonte, MG, Brasil

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 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

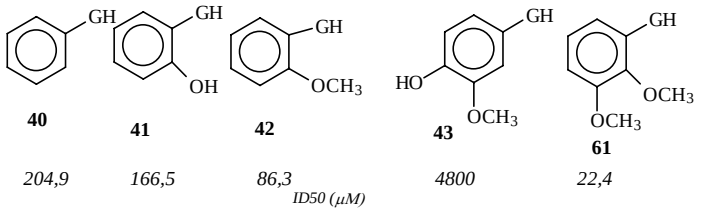
weight. Trypomastigotes isolated from untreated infected mice, as well as, 3h after treatment with BZ were evaluated at the molecular level, using flow cytometry. Total protein content (PC) was evaluated by the binding of fluorescein isothiocyanate (FITC); membrane-bound immunoglobulins (Ig) by FITC-conjugated 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

Borges MN/*, Figueroa-Villar JD
Departamento de Química, Instituto Militar de Engenharia, IME, Praça General Tibúrcio 80, Urca, Rio de Janeiro, Brasil *Instituto de Química da Universidade Federal Fluminense, Uff, Campus do Valonguinho, Niterói, RJ, Brasil

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*[3]. Molecular modeling study of these compounds with B-DNA showed that the drugs clearly preferred interaction with AT rich regions of the DNA[4]. The mechanism of action of these compounds is not yet know, neither the molecular mechanisms of guanylhydrazones interaction with *T.cruzi* membranes.



40 **41** **42** **43** **61**

204,9 *166,5* *86,3* *4800* *22,4*

ID50 (μM)

Figure I: GH= -C=NHNHC=(NH_2).HCl

In this work, we report the study of *T.cruzi* interaction between guanylhydrazones, Figure I, and membranes, using micellar systems as mimetic models. The study was performed using SDS and CTAB micelles. NMR techniques, spin-lattice relaxation time, ^1H T_1, 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
Lab.Biologia Celular, DUBC, IOC, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

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**). S*chemes* 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 10^4 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.

Supported by CNPq, Papes, Fiocruz.

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

Araújo CAC, Alegrio LV*, Castro D*, Lima MEF*, Leon LL
Departamento de Imunologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil
*Departamento de Química, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brasil

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 dirylheptanoids derivatives: des-o-metilcentrolobine (LD50=57㎜), the most active compound extracted from *C. sclerophyllum* and the methylated curcumin (LD50=5㎜). In the *in vivo* experiments several groups of Balb/c mice were injected subcutaneously with $3x10^6$parasites/50㎖. The drugs were injected in a concentration of 20mg/Kg weigth, 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 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.

Supported by CNPq, Faperj, Fiocruz.

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

Tempone AG, Lourenço CO, Spencer PJ, Andrade Jr HF*, Nascimento N
Divisão de Radiobiologia, Ipen,Cnen, SP, Brasil *Protozoologia, L IMTSP, FMUSP, SP, Brasil

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.L.amazonensis* promastigotes, using an *in vitro* assay. The Efficient Concentration 50% ($EC_{50\%}$) of the crude venom was determinated, 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.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.

Supported by CNPq , Rhae, LIMHCFMUSP.

CH-31
LEISHMANICIDAL ACTIVITY AND ULTRASTRUCTURAL CHANGES INDUCED BY SULPHUR SYNTHETIC NEOLIGNAN ANALOGUES

Pinto EF, Torres-Santos EC, Aveniente M*, Barata LES*, Massena VCA, Rossi-Bergmann B
Instituto de Biofísica Carlos Chagas Filho, UFRJ *Instituto de Química, Unicamp

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 biochemistry 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-SCl against *L. amazonensis* (IC_{50}=1mg/ml). In this study, the ultrastructural changes induced by LS-SCl 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-SCl synthetic analogues at 80 mg/ml. At the end of culture, the number of intracellular parasites was determined. Out of all substances tested, only LS-SCl was active, decreasing the intracellular parasite load by 94 %. To investigate the ultrastructural changes produced by LS-SCl on the parasites, *L. amazonensis* promastigotes were cultivated with 50mg/ml LS-SCl for 48 h and then processed for electron microscopy. LS-SCl 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-SCl 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-SCl 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

Oliveira DA*/***, Alario AF, Souza-Brito ARM**/***, Durán N*
Inst. Química., Biochem. Depart., Universidade de São Paulo *Inst. Química, Lab. de Biologia Química **Inst. Biologia, Depart. de Fiosiologia ***Fac. Ciências Médicas, Depart. de Farmacologia, Universidade Estadual de Campinas, C.P. 6154, Campinas, 13083-970 SP, Brasil

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 from *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 *Trichophyton rubrum* (5507 CCT strain), inhibiting the ergosterol synthesis from this dermatophyte (Oliveira et al. Mem Inst. Oswaldo Cruz 92, Supl.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 mmol/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.

Financial support: Padct, Finep, CNPq, Fapesp, Faep.

CH-33
MODELLING OF THE IRON SUPEROXIDE DISMUTASE FROM *TRYPANOSOMA CRUZI*: AN ALTERNATIVE FOR THE TREATMENT OF CHAGAS' DISEASE

Silva CHTP/*, Cabral CB, Garratt RC*
Instituto de Química de São Carlos *Instituto de Física de São Carlos, Universidade de São Paulo, Av. Dr. Carlos Botelho 1465, 13560-250 São Carlos, SP, Brasil

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 aproaches because a number of chelating agents and some of their derivatives have been as effectives 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 acive 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 essencial metal metabolism of *T. cruzi*, may lead to a new generation of pharmaceuticals for chemotherapic treatment of these diseases.

CH-34
MULTIDRUG THERAPY IN EXPERIMENTAL LEISHMANIASIS IN BALB/C MICE

Gonzalez AC, Sadigursky D, Sadigursky M
CPGM, Fiocruz, Escola Bahiana de Medicina e Saúde Pública e Faculdade de Medicina,UFBa, Salvador, Bahia, Brasil

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 Diclophenac - (5mg/mouse). 12 groups of 10 Balb/c were constituted for treatment and one group for control. Animals were infected with $1x10^7$ 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 Diclophenac or associated with Thalidomide alone or Sodium Diclophenac reducing the lesions in 50% or more. Cyclophosphomide an antimitotic drug, Thalidomide and Sodium Diclophenc did not had 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.

Supported by Pronex, CADCT, Capes.

CH-35
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

Vieira PP, Zalis MG, Oliveira R**, Pereira T*, Marques C*, Azevedo A*, Cerruti C*, Wirth DF***, Alwen A*
Laboratório de Biologia da Malária, Instituto de Biofísica Carlos Chagas Filho, UFRJ *USA Medical Research Unit, Walter Reed Army Institute of Research, Rio de Janeiro **Instituto de Biologia do Exército, Rio de Janeiro ***Harvard School of Public Health, USA

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 (pfmdr1 gene) was the cause of chloroquine resistance. However, genetic cross analysis between resistant and sensitive strains did not show segregation of mutations in the pfmdr1 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.

Supported by CNPq, NIH-NIAID.

CH-36
PANCREATITIS INDUCED BY SODIUM STIBOGLUCONATE (SB^V) IN BALB/C TREATED MICE AFTER INFECTION WITH *LEISHMANIA (LEISHMANIA) AMAZONENSIS*. COMPARISON WITH MEGLUMINE ANTIMONATE THERAPY

Vilhena V, Gomes RBB, Almeida MC, Freitas LAR , Barral A/*
Centro de Pesquisas Gonçalo Moniz,Fiocruz *Serviço de Imunologia, Hupes,UFBA, Salvador, BA, Brasil

Sodium Stibogluconate (Sb^V) and Meglumine Antimonate (Glucantime®) are the first choice drugs against all forms of Leishmaniasis. In Brazil, from 1996 to 1997 a Chinese formulation of Sb^V (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 Sb^V 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 10^6 promastigotes of the MHOM/BR88/BA/125 strain and divided in 3 groups: G1: 18 mice treated with 100 ml (10mg) Sb^V/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 Sb^V treated group. Nevertheless, mice treated with Sb^V 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 Sb^V. The inflammation had a focal aspect but in most cases was disseminated througout the organ. No pancreatitis was seen in mice of the Glucantimeâ treated and control groups.

Supported by Pronex, CADCT, Capes.

CH-37
PENTOXIFYLLINE AS AN IMMUNE MODULATOR DRUG ON THE EXPERIMENTAL CUTANEOUS LEISHMANIASIS (PRELIMINARY RESULTS)

Capp Neto M, Oliveira TS, Martins BJA, Rodrigues HA, Cardoso RG, Magalhães AV
Universidade de Brasilia, Departamento de Patologia, 70910-900 Brasília, DF, Brasil

In the animal model caused by *Leishmania (Leishmania) amazonensis* there are many linfokines 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 3.10^6 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 40^{th}, 80^{th} and 120^{th} day after the beginning of the treatment. Another group began the treatment with the drug 40 days after the inoculation, killed at the 80^{th} and 120^{th} 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 40^{th}, 80^{th}, and 120^{th} 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-a), modified the pattern of inflammatory response to the animal model under study. It reduced the macrophages propensity to vacuolation and yelled 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 TARSIUS* ARE POTENT TRYPANOCIDAL AGENTS

Carvalhaes Mara S, Silva Lindomar*, Bloch Carlos*
Laboratório de Imunoquímica, Universidade Federal de Goiás, R. Delenda Resende de Melo s/nº, Goiânia,GO, Brasil *Laboratório de Bioquímica e Química de Proteínas, UnB, Brasilia, DF, Brasil

We denoted that skin secretion of *P. tarsius* is extremely toxic to *Trypansoma 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

Torres MA, Echevarria A*, Canto-Cavalheiro MM, Cysne-Finkelstein L**, Temporal RM, Leon LL
Departamento de Imunologia, Instituto Oswaldo Cruz, Rio de Janeiro, RJ *Departamento de Química, Universidade Federal Rural de Rio de Janeiro, UFRRJ, Rio de Janeiro, RJ, Brasil **Depto. de Protozoologia, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

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 tecniques

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. Parallelly 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.

Supported by CNPq/Fiocruz.

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

Alves TMA, Soares CC, Alves RO*, Romanha AJ*, Zani CL
Laboratório de Química de Produtos Naturais *Laboratório de Parasitologia Celular e Molecular, CPqRR-Fiocruz, Av. Augusto de Lima, 1715, 30190-002 Belo Horizonte, MG, Brasil

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 2×10^6 trypomastigotes/mL. The plant extracts were assayed at a concentration of 500 µg/mL. Controls containing 2.5% DMSO or gentian violet at its IC_{50} (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.

Financial support: Pronex, Fiocruz, CNPq, IFS.

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

Dantas A, Ortigão M*, Traub-Cseko Y*, Polaquevitch PFC, Barbosa HS, De Castro SL
Laboratório de Biologia Celular, Departamento de Ultra-estrutura e Biologia Celular *Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, , 21045-900 Rio de Janeiro, Brasil

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* (Chan et al. *Antimicrob.Agents Chemother*. 37:1909,1993; Chan 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 b-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:

	Trifluralin/Y strain	Trifluralin/clone m28c	Chloralin/Ystrain
ID50/3 days (mM)	677.0±55.8	82.9±0.2	6.0±0.3

Ultrastructural analysis of parasites treated for 3 days with 100 mM **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.

Supported by Fiocruz, CNPq.

CH-42
THE EFFECT OF METOXY-AMIDINE ON *LEISHMANIA AMAZONENSIS* IS ASSOCIATED TO THE TRYPANOTHIONE REDUCTASE ACTIVITY

Gomes-Cardoso L, Echevarria A*, Aguiar-Alves F, Cysne-Finkelstein L**, Canto-Cavalheiro MM, Genestra M, Leon LL
Departamento de Imunologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Departamento de Química, Instituto de Ciências Exatas, UFRRJ, RJ ** Departamento de Protozoologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil

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 biossynthesis. 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 in our data, we decided to evaluate the effect of the methoxy-amidine on the activity of the trypanothine reductase of *L. amazonensis* promastigotes. The enzyme activity was followed up to 60 min in a mixture containing: 40mM HEPES, pH7.5, 1mM EDTA, 100mM 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.

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

Tinoco Luzineide Wanderley, Figueroa-Villar José Daniel
Departamento de Química, Instituto Militar de Engenharia, Praça General Tibúrcio 80, 22290-270 Rio de Janeiro, RJ, Brasil

Aromatic guanyl hydrazones have been shown to be promising agents for the prophylaxis of blood tainted with *T. cruzi* [1] and even for the chemotherapy of Chagas disease.[2] 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, [3] and more recently through an interaction of the drugs with the parasite membrane.[3] The NMR relaxation studies and the molecular modeling of the solution behavior of the guanyl hydrazones of 2-nitrobenzaldehyde (2NBGH, ID_{50} 87.5 mM), 3-nitrobenzaldehyde (3NBGH, ID_{50} 182.6 mM) and 4-nitrobenzaldehyde (4NBGH, ID_{50} 55.9 mM) showed that there is a direct relationship between the tendency of the drugs to form dimers with the values of ID_{50}. 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, T_1^S and T_1^{NS} 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 (t_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.

1-J. C. Messeder, L. W. Tinoco, S. L. de Castro, J. D. Souza, M. Santa Rita and J. D. Figueroa-Villar*, *Bioorg. Med. Chem. Lett.*, 1995, *5*(24), 3079.
2-O. A. Santos filho and J. D. Figueroa-Villar*, *Bioorg. Med. Chem. Lett.*, 1997, *7*(13), 1797.
3-M. N. Borges, D. Sc. Thesis Dissertation, Instituto Militar de Engenharia, 1998.

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

Melo MA, França-Silva JC*, Azevedo EO, Costa RT da*, Costa CA da**, Costa JO***, Mayrink W*, Genaro O*
Departamento de Medicina Veterinária, Universidade Federal da Paraíba, Campus VII - Fazenda Jatobá, Caixa Postal 64, 58700-000 Patos, PB, Brasil *Depto. de Parasitologia, ICB **Depto. de Análises Clínicas e Toxicológicas, Escola de Farmácia ***Depto. de Medicina Veterinária Preventiva, Universidade Federal de Minas Gerais, Caixa Postal 486, 31270-910, Belo Horizonte, MG, Brasil

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 antimoniate (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 x 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 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.

Supported by Fapemig, Capes-PICD, Fundação Banco do Brasil.

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

Santos Silva A, Oliveira Cardoso C, Cardoso CO, Zanqueta C, Bittar PCM, Lages Silva E, Ramírez LE
Departamento de Ciências Biológicas, FMTM, Uberaba, MG, Brasil

The aim of this study was to search, 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 (benznidazol, 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.

Supported by: CNPq (PIBIC 1997-1998)

CH-46
TREATMENT OF EXPERIMENTAL CUTANEOUS LEISHMANIASIS WITH TOPICAL FORMULATIONS OF PAROMOMYCIN

Gomes SFO, *Carvalho FAA, Fernandes APSM, **Tafuri WgL, *Gazinelli RT, *Tavares CAP, Ferreira LAM
Faculdade de Farmácia, Av. Olegário Maciel 2360, 30180-112 Belo Horizonte, MG, Brasil *Departamento de Bioquímica, Instituto de Ciências Biológicas **Departamento de Patologia Geral, Instituto de Ciências Biológicas Universidade Federal Minas Gerais, Belo Horizonte , MG, Brasil

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 5×10^6 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 hystological 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.

Supported by Fapemig and CNPq.

CH-47
TRYPANOCIDAL ACTIVITY AND CHEMOKINES MRNA EXPRESSION BY CULTURED MICE MYOCARDIAL CELLS INFECTED WITH *TRYPANOSOMA CRUZI*

Machado FS, Martins GA, Aliberti JCS, Cunha FQ, Silva JS
Departamentos de Imunologia e Farmacologia, Escola de Medicina de Ribeirão Preto,USP, 14049-900 Ribeirão Preto, SP, Brasil

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 can not 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-1b, IFN-g or TNF-a 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-1b, IFN-g and TNF-a 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.

Supported by Fapesp, Capes and CNPq.

CH-48
TRYPANOCIDAL ACTIVITY OF HYDROXYLATED DERIVATIVE OF N,N-DIMETHYL-2-PROPEN-1-AMINES

De Souza AO, Polaquevitch PFC*, Alderete JB/**, De Castro SL*, Durán N
Laboratório de Química Biológica – Bloco I-250, Instituto de Química, Unicamp, 13083-970 Campinas, SP, Brasil *Depto de Ultra-Estrutura e Biologia Celular, IOC/Fiocruz, RJ, Brasil **Depto de Química Orgânica, Universidade Concepción, Casilla 3-C, Concepción, Chile

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, ^{1}H-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 mM for (I) and 25.7 ± 1.7 mM 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
TRYPANOCYDAL EFFECT OF MULIN-11,13-DIEN-20 OICO ACID IN MICE INFECTED WITH TULAHUEN STRAIN OF *TRYPANOSOMA CRUZI*

Neira I, Sagua H, Castillo V*, Valladares A, Loyola A**, Bórquez J**
Unidad de Parasitología *Unidad de Hematología **Grupo de Química, Productos Naturales, Universidad de Antofagasta, Antofagasta, Chile

Chemoterapy of Chagas´s disease is still an unresolved problem. Treatment is only possible in the acute infection, few drug are avaliable and side effect have been observed. For these reason reseach and discovery of new compounds with trypanocidal activity are necesaries.

We have previously demostred that mulin-11,13-dien-20 oico acid, a new diterpenoid isolated from *Mulinun 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 10^6 trypomastigotes (Tulahuen strain). After that, 3 differents group 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 bleeded every 48 h and parasitemia curves and haematological aspect were studied.

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

Support: Specific Reseach Project (PEI A004) Universidad de Antofagasta.

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

Essodaigui M, Garnier-Suillerot A, Frézard F*
Laboratoire de Physicochimie Biomoléculaire et Cellulaire, (CNRS - 2056), UFR Biomédicale, Université Paris Nord, 74 rue Marcel Cachin, Bobigny cedex 93017, France *Departamento de Fisiologia e Biofísica, ICB, UFMG, C.P.486, 30161-910Belo Horizonte, MG, Brasil

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). Futhermore, 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

Delorenzi JC, Attias M, Andrade M*, Resende C*, Pinto AC*, Saraiva EMB
Laboratório de Imunobiologia das Leishmanioses, Departamento de Imunologia, Instituto de Microbiologia *Laboratório de Química de Produtos Naturais, Departamento de Química Orgânica, Instituto de Química- UFRJ, Rio de Janeiro, RJ, Brasil

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. Transmition 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 organele 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.

Financial support: WHO, Pronex, CNPq, Capes, Faperj.

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

Maldonado Rosa A, Fairlamb Alan H
Division of Molecular Parasitology and Biological Chemistry, University of Dundee, Dundee DD1 5EH, UK (ramaldonado@bad.dundee.ac.uk)

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 een 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. Preliminaries 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.

Supported by The Wellcome Trust (UK)
(1) Bringaud, F., Baltz, D. & Baltz, T. (1998) *Proc. Natl. Acad. Sci.* USA 95: 7963-7968.

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

Murta SMF/**, Ropert C*, Alves RO**, Gazzinelli RT/*, Romanha AJ**
Departamento de Bioquímica e Imunologia, ICB, UFMG, Caixa Postal 486, Belo Horizonte,MG, Brasil *Lab. Doença de Chagas **Lab. Parasitologia Celular e Molecular, Centro de Pesquisas René Rachou, Fiocruz, Caixa Postal 1743, 30190-002 Belo Horizonte, MG, Brasil

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 TNFa and RNI release by murine inflammatory macrophages primed with INFg. 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-a on induction of IFN-g 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-g 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.

Supported by CNPq, Fapemig, Papes/Fiocruz, Pronex Nº 2704.