QT

CHEMOTHERAPY
THE EFFECT OF MANNAN-COUPL ED ANTIMONY ON Leishmania (L.) chagasi INFECTED MACROPHAGES.

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Treatment of leishmaniasis has used antimonial, anfotericin B and petamidine. However, these drugs can cause several toxic side effects attributed to the high doses used and the interaction of the drugs with different host cells besides the macrophages which harbor the parasites.

Based on the presence of mannose receptors exclusively on the surface of macrophages antimony was coupled to a carrier, yeast mannan, as a trial of selective drug delivery to macrophages. The leishmanicidal effects of this complex (Sb-Man) were compared to those obtained with Glucantine, a pentavalent antimonial drug which has currently been used in the chemotherapy of leishmaniasis.

Mice peritoneal macrophages infected with L. (L.) chagasi were treated during 5 days with Sb-Man and 80% of the intracellular parasites were killed. A concentration 2 times higher of Sb used as Glucantine was necessary to obtain the same percentage of amastigote destruction. The viability of macrophages treated with Sb-Man was 98% as evaluated by Trypan blue exclusion. Infected macrophages cultures treated with PBS or free yeast mannan were used as negative controls.

The leishmanicidal effect of the antimony-mannan conjugate is presently being tested in hamsters infected with L. (L.) chagasi.

Supported by FAPESP.

THE ACTION OF GOLD SALT IN THE TREATMENT OF EXPERIMENTAL VISCERAL LEISHMANIASIS
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In 1989, Singh et al. treated 10 V.L patients resistant to gluconate with sodium aurothiomalate (SA), 6.94 mg/kg in alternate days with total dose of 250 mg. The purpose of this work is to evaluate the efficiency of the SA in hamsters.

GI - inoculated with parasites and treated immediately with SA (0,00027 mg/g hamster/day IM for 12 days. Controls animals inoculated with saline instead of parasites and treated.

GII - inoculated with parasites and treated 30 days after inoculation with SA with the same dose. The same control was used.

GIII - inoculated with amastigotes only. Controls animals were inoculated with saline. Fragments of liver, spleen, lung kidney were collected after 60 days of infection for histopathological studies as well as imprints of spleen and liver for parasite burden determination.

Histopathological changes observed in GI were: in the liver: hyperplasia and hyperroph of Kupffer cells, intense portal mononuclear infiltrate and inflammatory nodules in the parenchyma with many parasites (290,32 ± 77,05). In the spleen there was intense hyperplasia and hyperrophy of Mononuclear Phagocytic System (MFS) with light parasitism (97,87 ± 23,36), and depletion of white pulp. The lung showed a mild septal-thickening by mononuclear cells. The kidney had discreet interstitial inflammatory infiltrate, mild proliferation of mesangial cells and amyloid deposits. In the GII the histopathological changes found were similar to GI, with less intensity and the parasites burden was lower (Liver 90,48 ± 22,51 and spleen 24,92 ± 9,05). The GIII showed similar tissue changes like GI with liver parasite burden 145,62 ± 90,23 and in the spleen 45,72 ± 39,73.

These preliminar results suggest that SA exacerbates the infection in animals with gold treatment immediately after infection (P 0,0897). However when SA was showed the same histopathological changes.

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ON THE INHIBITORY ACTIVITY OF PLANT EXTRACTS IN THE DEVELOPMENT OF TRYpanosoma CRUZI IN WARREN'S MEDIUM.

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Assays were made with plant extracts obtained of roots, tubercles, leaves, and flowers of several species of the following plant families: Lauraceae (23), Aristolochiaceae (18), Composite (15), Moraceae (12), Bignoniaceae (6), Umbelliferae (14), Papaveraceae (4), Euphorbiaceae (3), Myrtaceae (3), Solanaceae (2), Labiateae (2), Cucurbitaceae (2), Nyctaginaceae (2), Annonaceae (2), Caryocaraceae (1), Rubiaceae (1), Myristicaceae (1), Verbenaceae (1), Cruciferae (1), to verify the inhibitory activity on the development of T. cruzi (y strain) in Warren's medium. The tests were made in quintuplicate with series of assays tubes containing 5ml of Warren's medium and 0.5 ml of culture using 10.10⁶ to 12.10⁶ flagellate forms. Ten days after the sowing, evaluation was made through a comparative observation on the development of the parasite forms in all tubes. Control tests with pure culture and the solvent were made. Sixty-seven (64.9%) of the tested extracts demonstrated a total or a partial inhibitory capacity on the development of culture forms of the protozoan.

HISTOPATHOLOGIC STUDY OF MURINE LEISHMAMIOTIC LESIONS AFTER PARENTERAL TREATMENT WITH KALANCHOE LEAF EXTRACT.

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We have previously observed that intraperitoneal injections with the crude extract of Kalanchoe pinnata (Kp) ("salão") leaves provokes regression of established cutaneous lesions caused in BALB/c mice by *Leishmania m. amazonensis.* To assess the effect of Kp on lesion histopathology and parasite load, mice were treated at the onset (days -3, -1, +1 and +3) and after establishment (days +45, +47, +49 and +51) of infection in the hind footpads. All the animals were killed at day +63 of infection and their whole feet were fixed in formalin-Millonig, decalcedified in 10 % EDTA in phosphate buffer (pH 7.0) and embedded in paraffin. Sections were stained with H & E, Toluidine blue and Lennert's Giemsa. The results showed that, in contrast to the untreated group, the lesions of animals treated 45 after infection presented a marked decrease in the macrophage and neutrophil infiltrate, an increase in lymphocytes and plasma cells, and presence of scattered epithelioid cells in small or large groups (tuberculoid-like lesion). Parasites were very scarce in vacuolated macrophages. In animals treated close to infection, the lesions exhibited different aspects, ranging from almost cured, with few parasites, to lesions similar to the untreated group, showing however, exacerbation of intramacrophage parasites. These data indicated that: a) Although not sterilizing lesions, Kp showed a "killing effect" by significantly reducing the number of parasites when administered after infection; b) Kp switches the lesion aspect from "lepromatoid" to "tuberculoid" type, suggesting stimulation of delayed type hypersensitivity (DTH); c) Kp may stimulate parasite growth when given at the onset of infection; and d) Kp treatment did not interfere with with the numbers of eosinophils and mast cells in the lesions.

Supported by CNPq.
THE DIRECT EFFECT OF KALANCHOE LEAF EXTRACT ON LEISHMANIA.

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We have observed that Kalanchoe pinnata (saião) leaf extract induces regression of cutaneous leishmanial lesions in mice (see accompanying abstract) in order to investigate the mode of action of Kalanchoe in leishmaniasis, the effect of various concentrations of the extract on the growth of Leishmania mexicana promastigotes and intracellular amastigotes was assessed in vitro.

Contrary to expected, addition of Kalanchoe to promastigote cultures accelerated parasite growth in a dose related fashion. With respect to amastigotes, it also promoted increased growth when added in smaller concentrations (30-60 μg/ml) to infected macrophages. At higher doses (500 μg/ml) however, there was an inhibitory effect, with no amastigotes found in cultures. We also observed that at higher doses there was a gross increase in the number of adherent macrophages, indicating that its leishmanicidal effect to amastigotes was not due to toxicity to the host cell, on the contrary, may have been due to macrophage activation.

There is evidence in the literature that cytotoxic CD8+ T cells can specifically lyse MHC-related infected macrophages, suggesting a role for these cells in leishmaniasis. We have found that Kalanchoe significantly increases the perforin production by a murine CD8+ cell line in vitro. We are now investigating the possibility that this increase in perforin production is accompanied by enhanced cytotoxicity to infected macrophages in a trial to explain its curing effect in vivo.

Supported by CNPq.

TREATMENT OF EXPERIMENTAL CUTANEOUS LEISHMANIASIS WITH KALANCHOE LEAF EXTRACT.

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Crassulaceae plants of the genus Kalanchoe ("saião") are popularly known in Brazil for their efficacy in the treatment of gastric ulcers, topical wounds, and cough. We have previously observed that 1 μg/ml leaf extract of K pinnata is sufficient to suppress the mitogenic responses of human and murine lymphocytes in vitro. To assess its immunomodulatory effect in vivo, we have used Leishmania m. amazonensis - infected BALB/c mice as a model.

Thus, mice were treated i.p. with a total of 16 mg of crude Kalanchoe extract at the onset and after the establishment of leishmanial lesions. We found that infections given at the time of parasite challenge did not change significantly the course of infection, but the group treated around the 45th day post-infection showed a sharp regression in the lesion growth, as compared with the rapid progression of lesions in saline - treated animals. These animals also showed a reduced DTH reaction to parasite antigen tested on the 50th day of infection, and slightly decreased levels in parasite-specific serum IgG measured on the 60th day. Lymphocyte proliferation to Con A in vitro was significantly lower in treated as compared to control untreated mice. The efficacy of various administration routes was compared, showing that peroral and i.p. were better than i.v. and topical routes.

These results indicate that the crude extract of Kalanchoe is effective in reducing leishmanial lesion growth and that this effect is accompanied by suppression of the immune responses.

Supported by CNPq.
ACTIVITY OF AN INTERMEDIATE OF VIOLACEIN BIOSYNTHESIS AGAINST Trypanosoma cruzi


Violacein, the major pigment of Chromobacterium violaceum has been found to exert trypanocidal activity (Haun et al. Biol.Res., in press, 1992). As cytotoxicity and genotoxicity studies to mammalian cells showed its highly toxic activity, several derivatives and intermediaries of violacem synthesis (3-[2,3-dihydro-5-[(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrorol-yliden]-1-3-dihydro-2H-indol-2-one) were prepared and assayed. The carbamidic acid (N-ethyl-(indol-3-yl)-2-indolethylamide) was active against T.cruzi at 400-600μM.

Cytotoxicity and trypanocidal activity of carbamidic acid (CB) and methylol violacem (M-Viol) - ID50/24h (μM)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Epimastigote</th>
<th>Amastigote</th>
<th>Trypomastigote</th>
<th>V-79 Cell</th>
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<tbody>
<tr>
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<td>46</td>
<td>-</td>
<td>12</td>
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<tr>
<td>M-Viol</td>
<td>Tul</td>
<td>28</td>
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<td>20</td>
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<tr>
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<td>750</td>
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<td>Y</td>
<td>-</td>
<td>256</td>
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<tr>
<td>Nif*</td>
<td>Y</td>
<td>10</td>
<td>100</td>
<td>47</td>
</tr>
</tbody>
</table>


Although information related to the carbamidic acid toxicity is still in a preliminary form, we have the chance to enhance its trypanocidal activity (as compared with violacem and methylol-vioacem), synthesizing new derivatives, as for example with a nitro group, responsible, at least in part, for the anti-T.cruzi effect of nifurtimox (Nif). Carboxamide appears as an excellent synthetic compound, in which any derivatization will be easier to perform than violacem. More detailed cytotoxicity and trypanocidal studies with carbamidic are currently under study.

SUPPORTED BY FAPESP, FINEP, PADCT, CNPq and IFS.

ACTIVITY OF 3-DIMETHYLAMINOPROP-1-ENE DERIVATIVES AGAINST Trypanosoma cruzi

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The chemotherapy of Chagas' disease remains an unsolved problem, and the search for alternative drugs continues. Only two nitroheterocyclic drugs are in clinical use, with severely restricted applicability for chronic patients, as well as being highly toxic. Aminopropane derivatives (DMP), which retain the double bond amine rearrangement of violacem (Haun et al., Biol.Res. in press, 1992) have been synthesized and assayed against different stages of T.cruzi.

We tested the isomers E & Z of 3-[4-bromo[1,1'-biphenyl]4-yl-3- (4-chlorophenyl)-N,N'-dimethyl-2-propen-1-amide as hydrobromide (BPA-1 E or Z and BPA-2 E + Z) and as free base (BPA-3 E or Z and BPA-6 E + Z). The ID50/24h for epimastigotes were 20.7±1.5, 20.0±3.0, 19.8±5.6 and 16.6±2.7 μM for BPA-1, 2, 3 and 6, respectively, with total lysis occurring at 40-50 μM for all drugs. The activity on bloodstream trypanopistles was lower than ID50/24h of 102.9±1.5, 131.5±18.5 μM for BPA-1 and 2, respectively. Total lysis of trypanostigotes was observed in the range of 200-300 μM, both at 48 and 28°C. Amastigotes, isolated infected macrophage line and grown axenically, showed a higher susceptibility to the four drugs than the two other forms of T.cruzi. Cytotoxicity assays for Chinese Hamster V-79 cells (Durán et al., Braz.J.Med.Biol.Res., 23:1303, 1990) showed ID50 of 12μM (BPA-1) and 10μM (BPA-2). Although nifurtimox exhibited a lower cytotoxicity (ID50=47μM) than DMP derivatives, these promoted similar trypanocidal activity as the nitroheterocyclic drug (Gonnert et al., Arzneim.-Forsch. 22:1582, 1972). Experiments are underway to analyse the effect of these synthetic compounds on parasite-host cell interaction and on experimentally infected animals.

SUPPORTED BY PADCT, FAPESP, CNPq, FINEP & IFS.
ACTION OF PENTOXIFYLLINE ON DEVELOPMENT OF TEGUMENTARY LEISHMANIASIS BY LEISHMANIA (LEISHMANIA) AMAZONENSIS
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Tegumentary leishmaniasis is characterised by a chronic inflammatory process with skin ulcer and cellular exudate constituted of histiocytes, lymphocytes and plasma cells with necrotic areas associated sometimes with giant cells. Experimental tegumentary leishmaniasis reproduces the human process.

The necrosis observed in tegumentary leishmaniasis has been interpreted as the result of an immune complex reaction when there exists little excess of antigen. The necrotic effect of this immune complex reaction possibly originates from lysozomal enzymes of neutrophils. However it is also possible that Tumour Necrosis Factor alpha (TNF-alpha) and Interleukin-1 (IL-1) participate in the inflammatory action and the instalation and development of the necrotic process. Recently it was found that Pentoxifylline also has a inhibitory action on the TNF-alpha and IL-1 leukocytes synthesis. The aim of the present trial is to verify the effect of Pentoxifylline on the necrosis occurring on experimental tegumentary leishmaniasis.

Forty eight C57BL/6Jb inbred mice were infected with 3x10^6 Leishmania (Leishmania) amazonensis promastigotes (MMOM/BR/76/Josefa strain), isolated from mouse lesions on the right ear. Two groups of 24 animals were defined - one was treated with 8mg/Kg/day 12/12 hs of Pentoxifylline ("Trental" - Hoechst) and the other was treated with Placebo (saline). After one week and for the successive eight weeks three animals from each group were sacrificed. The histopathological studies showed that the intensity of infiltrate is not modified between the groups but the presence of necrotic areas was reduced with the use of Pentoxifylline in the treated group. By blocking the inflammatory action of TNF-alpha and IL-1 on neutrophils that may influence an immune complex reaction it is possible that Pentoxifylline may diminish tissue damage in experimental tegumentary leishmaniasis.

The present model, that still needs to be improved, represents one more attempt to understand the biological basis of the tissue lesions in tegumentary leishmaniasis and constitutes another animal model for chemotherapeutic assays. Supported by CNPq, UnB and NIH grant AI 16282.

EFFECTS OF STEROID BIOSYNTHESIS INHIBITOR DRUGS IN Leishmania amazonensis
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(2) Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

Ketoconazole and Terbinafine are chemotherapeutic drugs that inhibit the biosynthesis of sterols such as the ergosterol present in the membranes of many parasitic fungi among other pathogens. Recent research indicates that these drugs may be useful weapons in the treatment of leishmaniasis. Nevertheless little is known about its biological effects at the cellular level. Therefore we decided to study the ultrastructure of Leishmania amazonensis promastigotes treated with different concentrations of Ketoconazole and Terbinafine for 3-4 days. The drug treatments produced remarkable structural alterations such as the appearance of multivesicular bodies (MVBs) in their cytoplasm. These MVBs could be observed in association with the Golgi apparatus and sometimes seemed to arise by vesiculation of other organelles such as mitochondria. MVBs could also be seen fusing with mitochondria and pericristaline arrays were observed in the mitochondrial matrix. Cytochemical detection of Acid Phosphatase (ACP) activity showed intense reaction products in these MVBs and also in membranes within the flagellar pocket. These reactions were both luminal and membrane-bound, suggesting the participation of both enzyme forms. Free membranes, in association with the cell surface also presented intense reactivity. Smooth endoplasmic reticulum membranes frequently formed myelin figures. Many large and sometimes polymorphic vacuolar granules were observed specially in Terbinafine-treated cells. Electron Spectroscopic Imaging (ESI) and Electron Energy Loss Spectroscopy (EELS) demonstrated the presence of phosphorous and oxygen and in these granules. In order to test whether these alterations could be reproduced in the parasites inside phagocytes vacuoles, we performed the same treatments with mouse macrophages infected for 3 days. Although vacuolized, the macrophages did not present effects similar to the ones observed in the parasites. Parasites were similarly affected by the drugs either intra- or extracellularly.

Supported by CNPq, FINEP and CEPG-UFRJ.
Plučea quit..<sub>3</sub>, COMPOSITAE: BIOASSAY GUIDED ISOLATION OF ACTIVE COMPOUNDS AGAINST Trypanosoma cruzi

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We have recently reported (CARDOSO, J.E. et al., 1990, XVII Annual Meeting on Basic Research on Chagas' Disease) that the crude ethanolic extract from P. quit.. sub>3</sub>, popularly known as "Quiticoca", is active against blood forms of T. cruzi. In order to monitor the isolation of the active compounds, we used a simpler, safer and faster assay based on growth inhibition of the epimastigote culture forms of T.cruzi at screening doses of 100 μg/ml. The plant was collected in the vicinities of Belo Horizonte, air dried, powdered and extracted sequentially with hexane and methanol. Only the hexane extract was active and it was partitioned between methanol-water (7:3) and ligroin followed by ethyl ether. The ether phase displayed trypanocidal and/or trypanostatic activity and was further chromatographed on a silica gel column by a step gradient resulting in 9 pools. Their activities varied from 27 to 96% of growth inhibition. The less polar pools were more active. Yellow crystals were obtained after concentration from the fourth pool (48% growth inhibition). This material was recrystallized from methanol and, based on spectroscopic evidence was shown to be Castacin, a known flavonoid. The IC50 for this compound was 62 μg/ml whereas for Nifurtimox, one of the trypanocidal drugs in clinical use, it was 1.7 μg/ml. After this crystallization the filtrate was subjected to successive column chromatography. It provided two other compounds which displayed 85 and 79% inhibition of parasite growth at 100 μg/ml. Spectroscopic data is being obtained for these compounds for their identification. Chromatographic fractionation of the other active pools are underway.

Supported by FIOCRUZ/CNPq.

SCREENING OF CRUDE EXTRACTS FROM COMPOSITAE SPECIES ACTIVE ON Trypanosoma cruzi

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We reported recently the trypanocidal activity of crude ethanolic extracts from 48 Compositae plants on blood forms of T. cruzi (CARDOSO, J.E., et al., 1990, Annual Meeting on Basic Research on Chagas' Disease). However, these assays make use of infective forms of the parasite, require animal handling and are time consuming. Thus, a simpler, safer and faster model suitable for mass screening and for guiding the isolation of active principles is desirable. The assay based on the growth inhibitory activity of culture epimastigotes of T. cruzi, Y strain, was found to fulfill these requirements. In brief, 8 mg of each extract were dissolved in 1 ml DMSO or ethanol. The parasites, at a final concentration of 10<sup>4</sup> epimastigotes/ml of LIT medium, were incubated in triplicate at 28°C for 4 days with the extracts at a final concentration of 100 μg/ml and with the controls Nifurtimox and Benzimidazole at their IC50. Parasite growth was then evaluated using an electronic cell counter and compared with that of the control cultures. Microscopic observation of the parasites was also performed in order to check for death, growth inhibition, motility and morphological alterations. Of the 82 Compositae species tested, 27% were highly active at the screening dose, inhibiting more than 80% of the parasite growth. Furthermore, while in the test with blood forms only 5 out of 48 extracts were active in the test with epimastigotes 17 were active. These results indicate a higher sensitivity of the test with epimastigotes, that could be due to a higher availability of the drugs or a higher sensitivity of the culture forms.

Supported by FIOCRUZ/CNPq.
AN EXPERIMENTAL AND CLINICAL ASSAY WITH KETOCONAZOLE IN THE TREATMENT OF CHAGAS DISEASE

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Ketoconazole, an azole antifungal drug already in the market has demonstrated to be active against Trypanosoma cruzi experimental infections (McCABE et al., 1983). We have now tested this drug in mice inoculated with 7 different T. cruzi strains and confirmed a diversity of cure rates similar to that observed with the standard drugs Benznidazole and Ketoconazole used clinically in chagasic patients. Moreover, we investigated in T. cruzi infected mice the presumable synergistic effect of the association Ketoconazole plus Lovastatin (an inhibitor of sterol synthesis) which displays an antiproliferative effect in vitro against epimastigotes and trypomastigotes yielded from axenic and tissue culture (URBINA et al., 1991). In our experiments Lovastatin was unable to potentiate the suppressive effect of Ketoconazole in T. cruzi infected mice. A group of 8 adult chronic chagasic patients was treated with Ketoconazole and clinically followed-up by one of us (J.R.C.) after informed consent. They were treated for 51 to 96 running days by oral route with doses usually indicated for the treatment of patients with deep mycosis (4.5 mg to 8.7 mg/kg). The cure evaluation was carried out in a variable period of 8 months to 5 years after treatment. The following tests were used: hemoculture in LIT medium, complement-mediated lysis, complement-fixation test, indirect immunofluorescence test and indirect haemagglutination test. From the 8 treated patients 6 surely failed to be cured as demonstrated by the positivity of hemocultures and serologic tests performed after treatment. In the remaining two cases in whom the hemocultures were not performed the positivity of the serological tests, particularly the complement-mediated lysis, strongly indicate persistence of T. cruzi infection. The identification of compounds already available in the market that by chance also demonstrate to be active against T. cruzi is one of the strategies which may be used to circumvent the lack of interest of the pharmaceutical industry in the chemotherapy of Chagas disease. The existence in the endemic area of some groups engaged on Chagas disease research favours that the whole process of experimental and clinical investigation be carried out.

Supported by CNPq

COMBINED IMMUNOTHERAPY AND CHEMOTHERAPY IN CANINE VISCERAL LEISHMANIASIS PRELIMINARY FINDINGS.

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In the present work we describe the preliminary results of a treatment by the combination of immunotherapy and chemotherapy in dogs naturally infected with Leishmania (Leishmania) chagasi. Five dogs captured by Fundação Nacional de Saúde in Araquari City (MG) were diagnosed serologically for visceral leishmaniasis (Complement Fixation Test - CFT, Indirect Fluorescent Antibody Assay - IFA and ELISA) and by the direct demonstration of the parasites in bone marrow aspirates (culture in NNN/LIT medium) and skin biopsies (printing smears). The dogs were submitted to a close scrutiny for all of the clinical features of CVL. Shortly thereafter, the animals were submitted to a skin test using Leishvac® as the antigen at a dose of 200μg of protein/animal (Genaro et al., 1992). Skin tests were negative for every animal. The vaccine was prepared with sonicated promastigotes of Leishmania (Viannia) braziliensis (MHOM/BR/74/C48), containing 6 mg of protein/ml. Immunotherapy was performed intradermally with two doses of 100 μl (600 μg of protein) of the vaccine with 3-month interval, associated with 500μg of BCG as adjuvant. One month after the first dose, we started chemotherapy with three series of meglumine antimoniate (Glucantime®). In each series, 60 mg/kg/day-1 of the drug were administered by intramuscular route during 15 days followed by a 10-day interval. Three months later and every two months thereafter, bone marrow and skin biopsies were performed for subsequent cultivation, murine films and printing slides, in search of amastigotes. The results show that: • Soon after the first series of Glucantime®, amastigotes were not found in the bone marrow and skin, remaining negative until presently (one year of observation); • Serum levels of IgG detected by ELISA and IFA decreased but did not disappear; • Skin tests remained negative in all dogs six months after the immunotherapy; • The dogs gained weight and the clinical signs disappeared four months after the initial treatment; • Electrophoretic patterns of the serum proteins showed reduction of the gammaglobulinemia to "normal" levels. Such features are compatible with the cure of the Leishmania chagasi infection, although a longer period of observation is needed for final conclusions. Further studies are in progress using immunotherapy to treat dogs with asymptomatic and oligosymptomatic CVL.

SUPPORTED BY FNS/FFB/CNPq/ UFMG.
QT-15

AN OPEN TRIAL TO EVALUATE THE TREATMENT OF AMERICAN CUTANEOUS LEISHMANIASIS USING LEISHVACIN® PLUS GLUCANTIME®, IN MINAS GERAIS, BRAZIL.

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An open trial was carried out on 100 patients living in an endemic area of American Cutaneous Leishmaniasis (ACL) aiming to compare chemotherapy and immunomodulation therapy for the treatment of ACL. In this area, most cases of leishmaniasis are caused by *Leishmania (Viannia) braziliensis*. All the patients had positive Montenegro skin test reactions and amastigotes were detected in printing slides of lesions. In the trial, one patient was treated with chemotherapy, the next was immunomodulation. Patients receiving chemotherapy were given 1ml/kg.day⁻¹ of Glucantime® IM, with a maximum dose of 10 ml/day. Patients receiving immunomodulation therapy were given a subcutaneous (flexor face of the forearm) dose of the vaccine (Leishvacin®). Biobras (Brazil) plus intramuscular Glucantime®. The initial dose of vaccine was 100 µL, increasing 100 µL daily until a maximum of 500 µL per day (this dose was used after). In both schedules, patients received daily injections for 10 days followed by an interval of 10 days without treatment, after which they were clinically examined to determine if further treatment was needed.

The following factors were analysed: age, occupation, weight, cure rate and persistence of disease, initial size of lesions, detection of parasites, size of Montenegro reaction, number of treatment series, total volume of antimony and total volume of vaccine. All the patients were cycled by both schedules and the analysis showed the following statistical differences:

- The number of treatment series was reduced by 41% using Leishvacin® plus Glucantime®.
- The amount of antimony employed was reduced by 54% with this therapeutic association;
- The mean cure time was 71.2 days with immunomodulation compared with 99.6 days for chemotherapy only.

The reduction in the number of series and consequently in the volume of antimony will reduce the time of treatment, the adverse effects to antimonial and the costs.

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MULTIDRUG RESISTANCE PHENOTYPE IN *Leishmania amazonensis* RESISTANT TO VINBLASTINE: REVERSAL BY CALCIUM CHANNEL ANTAGONIST

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We have previously demonstrated that *Leishmania amazonensis* cell line resistant to vinblastine is cross resistant to adriamycin, furthermore a DNA amplification of MDR-like gene was also observed in these cells. The aim of the present study was to investigate the reversal of resistance phenotype by verapamil, a calcium channel antagonist. *Leishmania amazonensis* promastigotes strain (IOCL 575) was cultivated in LIT medium supplemented with 12% fetal bovine serum. Resistant cell line was select in vitro by stepwise process until 100µM vinblastine (RV100). The EC₅₀ of wild type strain to verapamil was 4µM, whereas the EC₅₀ observed for RV100 cell line was 30µM, indicating an 8 fold resistance. Reversal of resistance phenotype was analyzed with verapamil concentration (5µM), which did not inhibited RV100 cell line growth. A growth inhibition of 11% was observed when RV100 cell line was incubated with vinblastine (100µM). In contrast, when RV100 cell line was incubated simultaneously with vinblastine (100µM) and verapamil (5µM) a growth inhibition of 58% was noted. The EC₅₀ of wild type strain to vinblastine was 12µM, while the EC₅₀ of RV100 cell line to this drug was 180µM, indicating a 15 fold resistance. Moreover, the EC₅₀ of RV100 cell line to vinblastine in presence of verapamil (5µM) was 80µM, indicating a 7 fold resistance. In conclusion, these results demonstrated a reversal of multidrug resistance phenotype in *Leishmania* cell line resistant to vinblastine, suggesting that the MDR genes may be involved in this phenomenon.

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TRANFUSIONAL CHAGAS' DISEASE IN PEDIATRIC PATIENTS
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We present 5 patients with chagasic infection acquired via transfusion. At diagnosis they were from 5 m to 10 years old. Criteria diagnosis were: a) negative mother serology; b) having been transfused; c) children should not have lived in endemic area; d) detectable T. cruzi in peripheral blood and/or reactive serology (titers > 1:16) by two technics. Nifurtimox in a dose of 10-15 mg/kg/d twice a day for 60-90 days was used as treatment. Negative serology was considered as evidence of cure.

Patient 1: Full term newborn, exanquinetransfusion due to blood incompatibility at 3 days. Sepsis signs. At 10 years old chagas diagnosis was made. Received treatment with persistent reactive serolouy in the follow up.

Patient 2: Full term newborn, exanquinetransfusion, sepsis signs. After knowing the donor was chagasic, diagnosis was made. Received treatment and in the follow up negative serolouy was observed (cure).

Patient 3: Transfused at 7 months, old at 11 months. Presented myocarditis. Received treatment and negative serologuy was observed (cure).

Patient 4: A six year-old child with myeloid leukemia, polytransfused, prolonged fever syndrome, sepsis signs. Received treatment and negative serologuy was observed (cure).

Patient 5: A 11 year-old child with humoral primary immuno deficiency, polytransfused, endocraneal mass biopsy T. cruzi amastigote. The aim of this communication is to warn about the possibility of finding pediatric patients with T. cruzi transfusional infection, especially in infants with immunodeficiency or immune system deficiency.

CONGENITAL CHAGAS' DISEASE: RESPONSE TO TREATMENT IN A NON-ENDEMIC AREA
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We have assisted 61 children with congenital Chagas' disease. The diagnosis criteria were: a) T. cruzi detection in peripheral blood by microhematocrit. b) In children older than 6 months detection the parasite and/or reactive serology (titers > 1:16) by two technics. c) Mothers with reactive serology d) Children should not have lived in endemic area e) not having been transfused. Nifurtimox in a dose of 10 - 15 mg/kg/d twice a day for 60-90 days was used as treatment. In the patients with positive parasitemia a serial parasite control was made to evaluate drug response. T. cruzi generally became negative between 15-20 days after beginning treatment.

In 41/61 children the treatment response was evaluated by serial control of antibody level. The disappearance of specific antibody was used as criterion of cure.

Considering the age of beginning treatment we observed negative serology in younger than 6 m in 18/19 (95%), within 7 m and 12 m 5/6 (83%), within 13 m and 36 m 6/7 (86%), within 37 m and 49 m 2/3 (66%) and in older 49 m 0/6 (0%).

Since treatment response is in direct relation with early beginning of treatment we suggest the early screening for congenital infection in chagasic mothers' children.
EVALUATION OF ORGANIC COMPOUNDS AGAINST BLOODSTREAM FORMS OF Trypanosoma cruzi

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Apart from the natural transmission through the insect vector, blood transfusion has an increasingly importance in the epidemiology of Chagas' disease. In this work we tested about 50 drugs between synthetic, semi-synthetic and natural compounds against trypomastigote forms.

In an initial screening, we considered as inactive the drugs that caused, after 24h, total lysis of the parasites above 1 mm, and as active those which lysis of the parasite occurred below 0.6 mm. Their effect upon mammalian cells were also analysed. The drugs were dissolved in DMSO, which final concentration was always below 0.5%.

Synthetic carbonic acid and hydroxylamine derivatives, nitrogenate heterocyclics, including pyrimidinic and pyrazinamide compounds and the natural compounds quinic acid-#21, dehydrocholic acid-#18 and the benzopyran catechin-#40 were inactive. Most of the synthetic hydroxylated benzenederivatives, as phenolic ones, were also inactive, with exception of #36—an halogenated phenolic derivative—and another aromatic compound dipikrilyl-#14.

Between those synthetic compounds considered active, we include: two phtalides—kresophytaline-#6 and naphtol phtalid-#32, two 1,4-oxo, azomethylenes—rosalic acid-#6 and an indophenol derivative, compound #43. Between active natural products we selected: the antraquinone derivative purpurin-#29, the benzopyran hematoxylin-#48, and agaric acid-#35.


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