XV REUNIÃO ANUAL SOBRE PESQUISA BÁSICA EM DOENÇA DE CHAGAS

QT

CHEMOTHERAPY
QT-1

NATURAL FLAVONOIDS – A NOVEL SERIES OF CHEMICAL COMPOUNDS ACTIVE AGAINST BLOOD FORMS OF TRYpanosoma CRUZI "IN VITRO"

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Transmission of Chagas' disease by blood transfusion is an important method of infection in South America (Brener, 1979). Colombiana, CL and Y strains of T. cruzi show different sensitivities to crystal violet and a new series of flavonoids, all of them isolated from Brazilian Leguminosae species. Eleven flavonoids were screened for assessing drug activity against infective blood trypomastigotes forms of T. cruzi "in vitro". Compound for testing were dissolved (or suspended) with DMSO and Krebs-Ringer glucose, mixed with an equal volume of parasite suspension. A parasite density of 2x10^5/ml was calculated for each test tube to controls and drug different final concentration from 2x10^-5 M to 3x10^-3 M. After 4°C/24 hours incubation the test were examined directly under the microscope. The susceptibility of C3H mice to T. cruzi infection was used to infectivity test. After the "in vitro" test the organisms/ml of blood with drug and controls were inoculated in mice, and were examined from day 7 to day 30. Serology by fluorescent antibody test to T. cruzi and hemoculture were carried in those mice which had not shown a positive parasitaemia in LIT medium at 28°C, the culture being observed after 3-4 weeks.

After all, all evaluation steps above mentioned we detected 3 out of the 11 flavonoids with high activity against T. cruzi blood trypomastigotes at the dose of 5x10^-4 M. The 3 active flavonoids, (3R)-claussequimone, (8)-4'-methoxydialbergone, (S)-4', 4'-dimethoxydialbergone, show a quinone moiety in their structure and deserve further investigation as potentially useful drugs in prevention of Chagas' disease transmission by blood transfusion.

CNPq and FINEP.

QT-2

ESTUDO DA ATIVIDADE DE EXTRATOS VEGETAIS SOBRE FORMAS SANGUÍCOLAS DO T. CRUZI.

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A considerável sobrevivência do Trypanosoma cruzi em sangue conservado a baixas temperaturas, faz da via transfusional o segundo mecanismo em importância epidemiológica para a transmissão da doença de Chagas, particularmente em áreas endêmicas. Desde a demonstração da atividade tripanosomicida do violeta de genciana em sangue armazenado, a sua utilização como quimioterapêutico se converteu em prática habitual em muitos bancos de sangue, apesar de seus efeitos colaterais que, de alguma forma, o distanciava do ser o fármaco ideal e justifica a necessidade de sua pesquisa de fármacos alternativos. Tendo em vista os resultados obtidos em testes de inibição do crescimento do Trypanosoma cruzi cultivado em meio de Warren, elaboramos um protocolo experimental para a avaliação da atividade de extratos vegetais sobre as formas sanguícolas do parasita. Assim, o sangue de camundongos infectados com amostras Y do T. cruzi está sendo testado frente aos extratos vegetais selecionados, em diversas concentrações em solvente inerte e avaliado quanto a sua capacidade de infectar lotes de animais jovens que, posteriormente, são acompanhados pelos artifícios clássicos da indicação de infeção aparente ou subpatente: níveis parasitênicos, cultura de sangue, xenodiagnóstico, parasitismo tissular e taxa de letalidade. Nestas avaliações estamos utilizando os extratos em séries com concentrações desde 10 a 300 ug, utilizando como controles o sangue contaminado puro, com violeta de genciana e com o sistema solvente.
ESTUDO DA ATIVIDADE DE EXTRATOS VEGETAIS SOBRE O DESENVOLVIMENTO DO TRYPANOSOMA CRUZI CULTIVADO EM MEIO DE WARREN.

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Com o propósito de se verificar a ação inibitória sobre o desenvolvimento do Trypanosoma cruzi (amostra Y) cultivado em meio líquido de Warren, testou-se 103 extratos alcoólicos obtidos à partir de raízes, tubérculos, ramos, folhas e sumidades floridas de exemplares pertencentes a 19 famílias de vegetais. Para tanto, determinou-se as concentrações alcoólicas não inibitórias do parasita e, a partir destes dados, elaborou-se as dissoluções dos extratos. Os testes foram realizados em séries com quintuplicatas em tubos contendo 5 ml de meio de Warren e 0,5 ml de cultura do Trypanosoma cruzi com cerca de 10 a 12 x 10^6 formas do parasita. Como controles utilizou-se de testes com cultura do flagelado puro e em soluço alcoólico. Na avaliação dos testes, realizou-se a leitura dos tubos no 10º dia através da contagem comparativa das variantes utilizadas. Os resultados obtidos nas diversas séries realizadas demonstraram ocorrência de inibição total do crescimento do flagelado em 33 extratos, ocorrência de inibição parcial em 27 extratos e ausência de atividade inibitória em 43 extratos testados.

BINDING OF CRYSTAL VIOLET TO TRYPANOSOMA CRUZI AND RAT LIVER MITOCHONDRIA

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Crystal violet has been shown to exhibit characteristics of an uncoupler of oxidative phosphorylation in isolated rat liver mitochondria (Moreno et al., J.Biol.Chem.,1988, in press), and in T. cruzi mitochondria in situ (Gadelha et al., submitted). We have determined the amount of crystal violet retained by mitochondria under various conditions by measuring the decrease of the dye concentration in the incubation medium containing either rat liver mitochondria or digitonin-permeabilized T. cruzi epimastigotes. About 50% of the dye was taken up by rat liver mitochondria de-energized by antimycin A and oligomycin either in the presence or absence of phosphate and the retention was not time-dependent. In contrast, the retention by these mitochondria energized with substrates was dependent on the incubation time. In the absence of phosphate the dye was taken up rapidly to a maximum of about 100% of the total dye after 15 sec and then decreased gradually. In the absence of phosphate, the dye was also taken up rapidly. However, about 30 sec after addition of dye, the dye began to be released to the level in de-energized mitochondria. About 60% of the dye was taken up by digitonin-permeabilized T. cruzi epimastigotes de-energized by antimycin A and oligomycin. In energized preparations the dye was taken up rapidly to a maximum of about 68% of the total dye in the absence of phosphate or 75% of the total dye in the presence of phosphate and the retention was not time-dependent. These results indicate different abilities of mammalian and T. cruzi mitochondria to take up and retain crystal violet that might be involved in the selective trypanocidal activity of this dye.

Supported by UNDP/WB/WHO Special Programme, and by CNPq and FINEP
EFFECT OF AMPHOTERICIN B ON T. cruzi AND ON ITS INTERACTION WITH HOST CELLS.
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Deptº de Ultraestrutura e Biologia Celular

Amphotericin B is a polyenic antibiotic with fungicidal activity, that increases the permeability of the cells by specific interaction with membrane sterols and has been used in some cases of cutaneous leishmaniasis. Earlier work of Abitbol and coworkers (1960, 61, 64) pointed out to the efficacy of amphotericin B (Anf. B) upon T. cruzi in experiments "in vitro" and with animals, and also, on a preliminary basis, on acute cases of Chagas' disease.

We investigated amphotericin B on our system (de Castro & Meirelles, Mem. Inst. Oswaldo Cruz 82: 209, 1987) analysing the effect upon the parasite and upon its interaction with host cells. Anf. B showed a potent effect on the proliferation of both amastigote and epimastigote forms, with an ID50 of 50 and 420 nM, respectively, after 2 days in axenic medium. This drug led to total lysis of trypomastigote forms, after 24 h/29°C, at the concentration of 500 nM.

The "treatment" of infected macrophage and heart muscle cell cultures gave no positive results, causing the drug, at 7.5 uM, severe damage to both host cells and, at 5 uM, having no effect on the intracellular parasites. However, in experiments with macrophages, we observed an inhibition of T. cruzi interiorization (interaction time: 1 hr) in the presence of Anf. B 10 and 20 uM. Using the higher concentration a slight alteration of the macrophages occurred, that was reversible after remotion of the drug.

Supported by FINEP, FIOCRUZ.

EFFECT OF PHENOTHIAZINES ON THE T. cruzi - HOST CELL INTERACTION
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Phenothiazines are widely used in psychiatric disorders and due to their cationic amphiphilic character, may interact with acidic phospholipids and calcium-regulated modulators, causing several physiologica alterations.

In a previous communication (Caxambu, 1987), we observed that chlorpromazine (CP) caused a dose-dependent inhibition of amastigote and epimastigote proliferation and electron microscopy analysis showed and intense vacuolization of the parasites. Now, we tested this drug and trifluoropromazine (TF) upon trypomastigote and both drugs led to total lysis of the parasite at 50 uM/24 hr/29°C. During interaction assays with heart muscle cells, CP and TF, in the range of 50 to 75 uM, caused an inhibition of amastigote proliferation on heart muscle cells, and at 60 uM, CP led to formation of numerous myelin figures on the host cell, possible due to their intralysosomal accumulation of polar lipids. In the experiments with peritoneal macrophages, TF 12.5 uM added after the interiorization step, similar to CP, led to an arrest of parasite proliferation. The remotion of the drug, 1 to 24 hr after the beginning of the infection, caused reversibility of the effect of both derivatives.

CP and TF are affective a gaisnt T.cruzi, causing a strong inhibition of intra-and extracellular proliferation of the parasite.

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TERAPEUTICA NA FASE CRÔNICA DA INFEÇÃO PELO T. CRUZI, COM O NIFURTIMOX E O BENZONIDAZOL.

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Foram submetidos à quimioterapia com o Bay 2502 (Nifurtimox), ou com o RO 7-1051, cinquenta camundongos suíços cronicamente infectados com diferentes cepas do T. cruzi de diferentes procedências porém previamente caracterizadas nos tipos biológicos: Tipo II e Tipo III. As cepas usadas eram provenientes de São Felipe, BA., Mambai, GO., Montalvânia, MG. e Bolívia. Vinte e um dos camundongos cronicamente infectados foram utilizados como controles não tratados. Os inóculos variaram entre/10.000 e 50.000 tripanomastigotas e a duração da infecção no início do tratamento era de 90 dias (fase crônica precoce) a 400 dias (fase crônica prolongada). O tratamento foi feito durante 90 dias nas doses de 200 mg/kg/dia durante 4 dias seguidas de doses de 50 mg/kg/dia para o Nifurtimox e de 100 mg/kg/dia para o Benzonidazol. Os resultados foram avaliados através dos testes de cura parasitológica (xenodiagnóstico, subinoculação e hemocultura) e de testes de imunofluorescência indireta em todos os casos além do estudo histopatológico dos animais sacrificados ao final do tratamento. Os resultados dos testes parasitológicos nos camundongos tratados com o Benzonidazol, mostraram 85,3% de negativização nos animais infectados com cepas de Tipo II e 43% com as cepas de Tipo III. Nos camundongos tratados com o Nifurtimox, observou-se 71,4% de cura parasitológica nos infectados com as cepas de Tipo II e 66% nos infectados com cepas de Tipo III. Quanto aos testes de imunofluorescência indireta, permaneceram positivos em 85,5% dos animais tratados. O estudo histopatológico mostrou nítida regressão das lesões miocárdicas e de músculo esquelético nos animais tratados e curados. Em 50% dos camundongos em que os testes de cura persistiram positivos, houve persistência de lesões histopatológicas discretas. Conclui-se que o tratamento na fase crônica pode determinar negativação parasitológica em percentagem elevada de casos, comparável aos obtidos na fase aguda com as cepas de Tipo II e mais elevadas com as cepas de Tipo III, com persistência da positividade da IFI.

Apoiado financeiro: CNPq e World Bank/UNDP/WHO-TDR.

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TREATMENT WITH NIFURTIMOX AND BENZONIDAZOL OF MICE INOCULATED WITH Trypanosoma cruzi DOES NOT AFFECT CONCANAVALIN A (Con A) RECEPTORS OF THE PARASITE BLOOD FLOWS (BTry)

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The binding of Con A (a lectin which specifically recognizes α-D-mannosides and α-D-glucosides) to T. cruzi blood forms (BTry) collected from infected mice treated with nifurtimox and benzonidazol was investigated to determine possible surface membrane alterations induced by the drugs. T. cruzi strains CL and VL-10, respectively sensitive and resistant to both drugs were used. The infected mice were treated with a single dose of 125 mg/kg of either drug and BTry collected 2 hours after treatment when a 30% decline in the parasitemia occurs. BTry were purified in ficoll-hypaque, deposited over slides, dried at room temperature and treated with fluorescein isothiocyanate (FITC) labelled Con A (2.9 moles FITC per mole Con A). The Con A reaction concentration varied from 500 to 1 µg/ml in serially half dilution way. The intensity of the fluorescence was determined in a Wild-Leitz immunofluorescence microscope. Experiments with the specific inhibitor of Con A, α-methyl-D-mannoside had also been carried out. Both strains express Con A receptors. VL-10 apparently has a lower number of binding sites for this lectin as demonstrated by the scattered punctiform fluorescence, whereas CL displays a more intense and diffuse fluorescence. The minimum Con A concentrations required to induce fluorescence in CL and VL-10 BTry were, respectively, 8.0 µg/ml and 16.0 µg/ml in both treated and control parasites. When the inhibitor was added (6.2 µg/ml) the minimum fluorescence inducing lectin concentrations for the CL BTry were 62 µg/ml for untreated parasites and 125 µg/ml for the treated; with the VL-10 strain these concentrations were, respectively, 125 µg/ml and 250 µg/ml. These results suggest that the active anti-T. cruzi nitroheterocyclic derivatives used in experimentally infected mice are not able to induce significant alterations in the parasite Con A surface receptors. Whether such data may exclude the participation of surface membrane lesions in the mechanism of parasite clearance in treated animals is still being investigated.

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QT-9

DRUG CROSS-RESISTANCE IN Trypanosoma cruzi STRAINS

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Cross-resistance to non-related drugs has been detected in Plasmodium falciparum and Leishmania. The low percentag es of observed in chagasic patients may also result of the natural resistance of T. cruzi strains to the two drugs used clinically (nifurtimox and benznidazol) (FILARDI & BRENER, 1987, Trans. R. Soc. Trop. Med. Hyg., 81: 755-759). In order to investigate the occurrence of T. cruzi resistance to different non-related drugs, groups of mice had been inoculated with 10⁴ blood forms of 5 parasite strains (Colombiana, Noel, SC.28, VL-10, Yopu) resistant to benznidazol (N-benzyl-2-nitro-1-imidazolacetic) and nifurtimox (3-methyl-5'(nitrofururylidene-amino)-tetrahydro-4H-1,4-chiazine-1,1-dioido). At the 4th day of infection the animals inoculated with each strain were divided into 5 groups and treated by oral route respectively with the following drugs: nifurtimox (100 mg/kg, 20X), benznidazol (100 mg/kg, 20X); megazol [2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole] (100 mg/kg, 20X); NK-436 [3-(1-methyl-5-nitroimidazol-2-yl)-3a,4,5,6,7a-hexahydro-1,2-benzoxazole] (100 mg/kg, 20X); Ketocanazol [2-6(R)-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-](S)-ylmethyl> - -(2-2-(4-chlorophenyl)-ethyl)=1,2,3,4-tetra-hydro-isquinolinol-6-yl)-ether, used clinically as a local and systemic antifungi agent. Cures were ascertained by negativization of hemoculture in LIT (liver infusion-tryptose) performed 30-60 days after treatment and indirect fluorescence test carried out 6 to 9 months also after drug administration. Results are shown in the following table:

<table>
<thead>
<tr>
<th>T. cruzi strains</th>
<th>Benznidazol</th>
<th>Nifurtimox</th>
<th>Ketoconazole</th>
<th>NK-436</th>
<th>Megazol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colombiana</td>
<td>6.6</td>
<td>0.0</td>
<td>7.6</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Noel</td>
<td>3.4</td>
<td>3.4</td>
<td>42.8</td>
<td>66.6</td>
<td>78.5</td>
</tr>
<tr>
<td>SC.28</td>
<td>3.5</td>
<td>0.0</td>
<td>80.0</td>
<td>100.0</td>
<td>86.6</td>
</tr>
<tr>
<td>VL-10</td>
<td>20.0</td>
<td>7.6</td>
<td>6.6</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Yopu</td>
<td>3.3</td>
<td>6.6</td>
<td>60.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

If an arbitrary criterion of 50% cure is selected for identification of resistance as suggested by FILARDI & BRENER (1987), cross-resistance in relation to Ketoconazole was detected in at least two of 5 T. cruzi strains already demonstrated to be highly resistant to the nitroheterocyclic derivatives benznidazol and nifurtimox. **FINANCIAL SUPPORT BY WHO, CNPq AND FINEP.**

QT-10

LONGITUDINAL STUDY OF ANTI-T._cruzi IgM, IgG AND LYTIC ANTIBODIES IN PATIENTS WITH ACUTE CHAGAS' DISEASE DURING AND AFTER SPECIFIC CHROMOTHERAPY.


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Twenty six individuals developed acute Chagas' disease following a meeting at rural region in Paraíba. The source of infection seems to be a beverage contaminated with mammal secretions. The kinetic of IgM and IgG anti-T._cruzi antibodies determined by indirect immunofluorescence and the presence of lytic antibodies to bloodstream trypanomastigotes were analyzed in sera of twenty-three chagasic patients followed for twelve months.

The table shows the percentage of patients with the reciprocal of serum dilution and the geometric mean in different intervals.

<table>
<thead>
<tr>
<th>Duration of disease (month)</th>
<th>Number of Patient</th>
<th>IgM negative</th>
<th>IgG negative</th>
<th>Geometric mean</th>
<th>Geometric mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤ 40</td>
<td>&gt; 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>101</td>
<td>40</td>
<td>50</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>32</td>
<td>21</td>
<td>47</td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>65</td>
<td>18</td>
<td>17</td>
<td>0.5</td>
</tr>
<tr>
<td>12</td>
<td>23</td>
<td>91</td>
<td>0</td>
<td>9</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* Percentage of patients at each time interval.

Sera tested at one and two months of infection could not be analysed for the presence of lytic antibodies because the sera promoted reduction in the number of bloodstream trypanomastigotes in the absence of complement as already observed by Stefani et al. (1996).

Lytic antibodies were determined in samples collected at five and twelve months after infection. All sixteen samples analyzed at the fifth month were negative. At one year evolution, twenty one samples were negative and two presented doubtful results. One of these samples also showed positive IgM antibody.


Supported by Laboratórios de Investigação Médica - Hospital das Clínicas -PMSP.
STUDY OF THE ACTION OF Porphyrins in Trypanosoma cruzi AND Leishmania amazonae.

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The action of porphyrin derivatives was studied in two levels: a) in the mobility, growth, and oxygen uptake of culture forms of Trypanosoma cruzi and of Leishmania amazonae and b) in tissue culture infectiveness of bloodstream forms of T. cruzi.

Hematoporphyrin derivatives (HpD) have been extensively studied due to its importance in the diagnosis and treatment of malignant tumors. Among parasites, bloodstream forms of Trypanosoma brucei were found to be lysed in vitro by the addition of hemol and zinc. Hematoporphyrin D cured mice infected with Trypanosoma brucei brucei.

In the present study, we found that treatments with HpD plus light immobilized trypomastigotes and epimastigotes of T. cruzi and promastigotes of L. amazonae. Infectiveness to Vero cells showed that trypomastigotes preincubated with HpD and submitted to light for 6 min. were able to cause a very small infection (below 10%). Oxygen consumption was dramatically increased by all parasite forms in presence of HpD. Also, electron micrographs showed drastic alterations in the nuclei and ketylplasts of both protozoa.

Supported by CNPq, FINEP, CEPG-UFRJ.


A bioassay has involved l. amyris that was obtained as major compound from hexane extract of M. sericea's wood. The residue was extracted with ethanal and part of it was acetylated (AC/O, 48 hr). The hexane extract on concentration under reduced pressure yielded a residue and the solid was eluated in chromatography column (Sigel) with hexane - chloroform (9:1). The percolate on removal of the solvent mixture, gave four compounds and the l. amyris was crystallized in methanol, characterized by m.p. 1977-1998 and spectroscopic methods (I.R, mass, R.M.N. H and R.M.N.13 C) (Graulf et al., 1986).

It has been studied two crude extracts (ethanolic and acetylated) and one substance (l. amyris). Different concentrations of drugs incubated in vitro with 105 viable l. braziliensis promastigotes (Table I). The parasite growth on culture media was made on a newbauer chamber. The result of two repeated experiments have showed that both the two extracts and the pure substance has had antileishmanial activity (Table II). Although the best results have been obtained with l. amyris which showed a great active and inhibited parasite growth on shorter time.

At present, l. amyris has been tested as antileishmaniasis in vivo against l. mexicana amazonensis by topical and intraperitoneal inoculation on S/alb/c mice.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NATURAL PRODUCT</strong></td>
<td><strong>CONCENTRATION</strong></td>
</tr>
<tr>
<td>B. amyris</td>
<td>0.050 mg/ml</td>
</tr>
<tr>
<td>B. amyris (half)</td>
<td>0.025 mg/ml</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.050 mg/ml</td>
</tr>
<tr>
<td>Ethanol (half)</td>
<td>0.025 mg/ml</td>
</tr>
<tr>
<td>Acetylated</td>
<td>0.200 mg/ml</td>
</tr>
<tr>
<td>Acetylated</td>
<td>0.100 mg/ml</td>
</tr>
<tr>
<td><strong>NATURAL PRODUCT</strong></td>
<td><strong>DAYS</strong></td>
</tr>
<tr>
<td>B. amyris</td>
<td>1.0000</td>
</tr>
<tr>
<td>B. amyris (half)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.0000</td>
</tr>
<tr>
<td>Ethanol (half)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Acetylated</td>
<td>1.0000</td>
</tr>
<tr>
<td>Acetylated (half)</td>
<td>1.0000</td>
</tr>
<tr>
<td>Control</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

8alb/c mice.
The effect of meglumine antimonials was determined on the growth of 14 Leishmania isolates: L. b. braziliensis (MHOM/BR/75/M2903), L. b. panamensis (MHOM/PA/71/L894), L. a. amazonensis (IFLA/BR/75/A148) and L. b. mexicana (MHOM/BZ/82/SEL 21) as reference strains; L. b. braziliensis (LTB 259), L. b. braziliensis (LTB 260), L. b. braziliensis (LTB 299) and L. b. braziliensis (LTB 320) isolated from patient; L. b. braziliensis (MN5/BR/85/M 9554), L. b. braziliensis (ISO/BR/85/M9947), L. b. guyanensis (IUB/BR/85/N9957), L. b. guyanensis (UIMB/BR/85/M9945), L. m. amazonensis (IFLA/BR/86/M 10995) and L. m. amazonensis (IFLA/BR/85/M 10996) isolated from phlebotomine sandflies or reservoir animals on Para state. The promastigotes were allowed to multiply either in the absence of the drug or in its presence (concentration of 16,4 μM) in a complex liquid medium with 40% of whole blood rabbit (Figueiredo et al. 1976). Rev. Inst. Med. Trop. São Paulo 18:306-314).

At intervals of incubation of 24 hours at 250C the cell number were measured with a Coulter Counter. Growth inhibition varying from 0 to 95% was observed among the Leishmania strains in the presence of the drug, after 96 hours of incubation. A criterium for the resistance/sensitivity of the strains to the drug was established based on values of standard deviation/growth inhibition smaller than 40% was considered as an indication of resistance to the drug action. Growth inhibition greater than 40% indicates sensitivity. Using this criterium the strains can be classified as follows: the strains LTB 320, LTB 299, LTB 260, M 2903, M 9954, M 9957, M 9945 and M 10996 are sensitive to antimony, while LTB 259, M 9947, M 10995, L5 94, PNB and SEL 21 are not. Among the sensitive strains LTB 259 and LTB 320 showed the highest sensitivity, with growth inhibition of 93 and 95%, respectively. Besides that, comparison of the growth curves revealed alteration in the growth pattern of some strains, which showed either delayed beginning of the logarithmic phase (LTB 299) or increase duration of this phase (LTB 299, LTB 259 and M 9947).

The possibility that antimonial resistance in Leishmania is due to a failure of the drug to reach toxic levels within the parasite due to acquired enhanced active efflux, the effect of verapamil (2 μM), a calcium channel blocker which reverses the drug resistance of cultured neoplasms, the antimony-resistant Plasmodium falciparum by similar mechanism, was evaluated. The tests were performed with strains M 9947 and LTB 259, in the presence and in the absence of meglumine antimonials. Preliminary studies indicate that verapamil alone or associated with antimony has no antileishmanial properties "in vitro" since no effect on the growth curve was observed. Supported by CPq-AFME.

**Slow Dose Antimonials Therapy in American Cutaneous Leishmaniasis - A Double Blind Study.**

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In a previous Congress (Coombs, 1987) the AA presented two communications (Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. Vol. 82, November 1987 pg 176 abstracts QT-11 and QT-12) about the treatment of American Cutaneous Leishmaniasis (ACL) with low doses of antimonials. In these open studies patients follow-up extended from 1 to 5 years. The cutaneous lesions were completely healed and no mucosal involvement was detected.

In order to prove the efficacy of a low dosage treatment, a double-blind, comparative study between high and low doses of antimony was proposed. High and low doses were respectively 20 and 20 mg of N-methyl-glucamine antimoniate per kg/day during 30 consecutive days.

From September 87 to February 88, 22 patients (13 males and 9 females) were studied: 19 from the metropolitan region of Rio de Janeiro city or its immediate vicinity and 3 from the states of Ceará, Minas Gerais and Para. Age varies from 11 to 65 years. Diagnostic criteria included the clinic appearance of the lesion, the epidemiologic notion and a positive Montenegro test. Parasites were detected (by culture, histopathological examination and/or smear) in 18 patients.

Patients were casually distributed in two groups of 11 individuals each. The doses were administered by intravenous route, diluted in distilled water allowed in the same final volume of 30 ml. Distribution of high and low doses and the preparation of the syringes for the application were in charge of the Chief Nurse alone, in order to preserve the seal over the dosage employed.

After a 30 days series the patients were examined by the AA, in order to certify the clinical cure of the lesion and to search for maximal lesions. Follow-up of the patients continues each month until present time.

6 months after last patient's end of treatment the study was opened.

**Results:**

At the end of the 30 days series, 16 patients (70.7%) presented healed lesions (7 with high and 9 with low dosage). In 6 patients (27.3%), clinically active lesions were still present after 30 days (4 with high and 2 with low dosage). Not healed lesions were always situated on the legs and/or feet. Of these 6 patients, 3 (one with low and the other with high dosage) had their leg lesions healed 20 and 70 days, respectively, after the end of the 30 days series. No other treatment was employed. In another 2 patients (one with low and one with high dosage) with lesions on the legs and in other locations, the former remain healed but the latter were cured after the end of the treatment. The last 2 patients have abandoned the study.

**Conclusions:** Our study strongly suggest that there is no difference between high and low dosages of antimonials for the treatment of ACL. With both dosage schedules cicatrization of ulcers occurs completely after a 30 days series in 72.7% of cases. In the remaining 27.3% not healed lesions were located on the legs and arms as a disturbing factor. It is interesting to note that in patients with lesions on the legs and on other locations, only the leg lesions remains unhealed by the end of treatment this fact occurring either with high and low dosages. As we could stated in previous studies patients with a low dosage rarely remain well and with no mucosal involvement after a 5 years follow-up. The advantages of a small dose are obvious, viz. reducing the side effects and being more economical. The use of amphotericin B with all capacity would be desirable.
OPTIMIZATION OF THE GLUCANTIME SCHEDULE FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS CAUSED BY Leishmania (viannia) braziliensis.
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Recommendations for the treatment of cutaneous leishmaniasis with Glucantime vary from 17-28 mg Sb/kg/day for 10 continuous days (Rhodia package insert) to 20 mg Sb/kg/day for 20 days (SUCAM). Apparently these schedules were derived from clinical experience, as no controlled studies have been published. The objective of the present study is to optimize the glucantime schedule for treatment of cutaneous lesions caused by L. braziliensis using a double blind protocol with strict compliance. The study was conducted at Corte de Pedra, Bahia. Patients with ulcers consistent with cutaneous leishmaniasis and a positive Montenegro skin test were entered into the study on a voluntary basis. Leishmania were demonstrated in 85% of the lesions. The patients were randomly treated with 10 or 20 mg Sb/kg, daily for 20 days. The glucantime was mixed with 5% dextrose solution to a volume of 20 ml and injected intravenously. All lesions showed signs of healing on the last day of treatment with loss of the infiltrated border around the ulcer and initial re-epithelization (RE). At 6 weeks most lesions continued to re-epithelize and scar. Results from the 12 week examination are shown:

It is important to note that >90% of the lesions with re-epithelization at 6 weeks improved or cured by 12 weeks indicating that healing was induced by glucantime.

The influence of spontaneous cure was probably minimal as 40% of local patients heal without treatment within 6 months.
The frequency of side effects (arthralgia, myalgia) was higher in the 20 mg group, but they were not serious enough to stop treatment. Since the efficacy of the two schedules were similar, the lower dose is preferred to minimize side effects. However, long term evaluations are required to determine the frequency of relapse and metastasis to the naso-oral mucosa before firm recommendations can be made.

EFFECTS OF PHOTORADIATION THERAPY WITH HEMATOPORPHYRIN DERIVATIVE ON LEISHMANIA MEXICANA AMAZONENSIS.

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Hematoporphyrin derivative (HpD) localizes selectively in malignant and rapidly metabolizing tissues and undergoes a cytotoxic reaction when exposed to light of a specific wavelength.

In the present study we found that treatments with HpD plus light immobilized promastigotes of L. mexicana amazonensis and increased oxygen consumption.

Also, preliminary results of our laboratory showed that the combined effect of HpD and activating red light was responsible for "in vivo" antiprotosoal activity. Infected hamsters showed, after one month treatment, a partial healing in the footpad and no secondary infection with other microorganisms as compared with control animals, where continuous lesions were observed.

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IN INVOLVEMENT OF CYSTEINE PROTEINASES IN THE DESTRUCTION OF LEISHMANIA MEXICANA AMAZONENSIS AMASTIGOTES BY AMINO ACID ESTERS

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Lysozymotropic amino acid esters can destroy intracellular and isolated Leishmania mexicana amazonensis amastigotes by a mechanism postulated to involve trapping of the compounds and their enzymatic hydrolysis within acidified lysosome-like parasite organelles (Rabinovitch and Alfieri, Braz. J. Med. Biol. Res. 20: 665, 1987). Three findings support the role of amastigote enzymes in the killing by the esters: a) the specificity of the amino acid moiety, exemplified by the activity of leucine methyl ester (Leu-OMe) and the inactivity of the structural analogue Ile-OMe (Rabinovitch and cols., J. Exp. Med. 163: 520, 1986); b) the protection conferred by Ile-OMe and other esters against damage by Leu-OMe (Alfieri and cols., Parasitol. 95: 31, 1987); c) the demonstration that the proteinase inhibitors antipain and chymostatin protect amastigotes from damage by Leu-OMe and certain other esters (Alfieri and cols., Mol. Biochem. Parasitol. 29: 191, 1988).

The present experiments provide evidence for the involvement of histone proteinase activities in the leishmanicidal activity of amino acid derivatives. After electrophoretic separation of total parasite extracts in SDS-polyacrylamide gels containing copolymerized gelatin, L. m. amazo- nensis amastigotes were shown to contain several proteinase activities, distributed in the 25 to 33 and 70 to 100 kDa regions of the gels. The activities associated with the low molecular weight enzymes were abrogated by 5 μg/ml antipain, chymostatin or leupeptin, as well as by the specific and irreversible cysteine proteinase inhibitors E-64 (2 μM) and Z-Phe-AlaCH2N2 (5 μM). Gelatin digestion by the low molecular weight enzymes was selectively inhibited by 40 mM Leu-OMe or Ile-OMe, as well as by the benzyl esters of Leu, Ile and Gly (10 mM). Furthermore, stronger inhibition was obtained with 5 mM of the dipeptide ester Leu-Leu-OMe. The results suggest that amastigote cysteine proteinases are the targets for the amino acid derivatives.

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USE OF RECOMBINANT HUMAN INTERFERON-GAMMA COMBINED WITH PENTAVALENT ANTIMONIAL (Sbv) ON THE THERAPY OF VISCERAL LEISHMANIASIS.

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Immunological dysfunction is an important aspect of the visceral leishmaniasis. Several defects have been described including the absence of gamma Interferon and interleukin 2 production during the active disease. The failure of the current therapy (Sbv) for visceral leishmaniasis is about 15%. The alternative drug is amphotericin B which have high toxicity and it is not fessable to use for all patients. Recently with the availability of the recombinant cytokines for use in clinical trials, we evaluate the useful of a combination of recombinant human interferon gamma (rHuIFN-gamma) plus pentavalent antimony (Sbv) to treat patients with refractory visceral leishmaniasis and others with severe ill form of the disease. The rHuIFN-gamma at a dose of 100ug/m2/IN daily as combined with Sbv in a dose of 20mg/kg/day during 10-20 days. The trials were carried out into two groups: group A: six patients who failed to respond to several courses of pentavalent antimonial alone. Group B: nine patients with severe manifestation of the disease. The criteria for the diagnose and the control of the therapeutic response in both groups was made by the demonstration of viable leishmania searched by splenic aspiration punctation. In the group A only 2 of 6 patients did not respond to the first 10 days course of combined therapy and required an extra 20 days therapy to decrease the parasitism degree of the spleen. From the 9 patients of the group B, only one required an extra course of combined therapy. The clinical follow up show showed that the signs and symptoms dramatic disappeared during the therapy, and the immune response to L.dopovani antigen was restored earlier than we expected. The combination therapy was well tolerated. Fever was the ony side effect noted during the gamma interferon therapy. We conclude that the use of rHuIFN-gamma plus Sbv it is a new potential therapy for visceral leishmaniasis mostly for resistant cases.

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