QM-1 – *BOTHROPS MOOJENI* VENOM KILLS *LEISHMANIA SPP.* PROMASTIGOTES BY HYDROGEN PEROXIDE GENERATED BY ITS L-AMINO ACID OXIDASE

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Leishmaniasis include a spectrum of human infectious disease, ranging from self-healing cutaneous ulceration to a progressive and lethal visceral infection. The disease is estimated to affect about 12 millions of people, with 400.000 new cases worldwide per year. The first-line drugs for Leishmaniasis treatment, remains on pentavalent antimonial, since their discover in the beginning of the century. Despite of the high toxicity and the cumbersome schedule, many reports discusses clinical resistance and treatment failures. Venoms of numerous land snakes posses cytotoxic or lytic effects on tumor cells *in vitro*. Growth inhibition of *Trypanosomatidae* by snake venoms was described, but no attempt to purify the active fraction was done. In this work, we demonstrate that *Bothrops moojeni* venom has a killing effect *in vitro* against *Leishmania spp* promastigotes, determined by cellular viability assay, using oxidative conversion of MTT. This activity was sequentially purified from crude venom by molecular exclusion and ion exchange chromatography. When eluted fractions were tested, anti-*Leishmania* and L-amino acid oxidase (L-AAO) activities coeluted in the same fractions. The molecular weight of the enzyme was estimated to be 140kDa by molecular exclusion chromatography, and 69kDa, by SDS-PAGE, migrating as a single band, with an isoeletric point of 4.8, determined by isoelectric focusing. This 135-fold purified L-AAO of *B.moojeni* venom is an acid enzyme with homodimeric constitution, active against *Leishmania spp* promastigotes from New World, with an efficient concentration of $EC_{50\%}$ =1.80 µg/ml against *L.(L).amazonensis*, $EC_{50\%}$ 0.78 µg/ml against *L.(V.)panamensis* and $EC_{50\%}$ 0.63 µg/ml against *L.(L.)chagasi*. Ultrastructural studies of promastigotes affected by L-AAO, demonstrated cytoplasmic death, with edema in several organelles, as mitochondria and nuclear membrane, before cell disruption. The action of L-AAO was demonstrated to be hydrogen peroxide-dependent, by abolishing the killing effect by catalase, absence of substrate or by showing similar effects by added hydrogen peroxide. These data could help in the development of alternative therapeutic approaches in leishmaniasis treatment.

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QM-2 – AQUEOUS EXTRACT OF *KALANCHOE PINNATA* PLANT UP-REGULATES TH1 CYTOKINES IN NORMAL AND *LEISHMANIA* - INFECTED MICE

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We observed previously that the aqueous extract of *Kalanchoe pinnata* (Kp) administrated by oral route controlled lesion of Balb/c mice infected with *Leishmania amazonensis* (Da-Silva et al, *Acta Tropica*, 60: 201-210, 1995). The protective effect of Kp in leishmaniasis was related with immunossupression (Rossi-Bergmann et al, *Phytoterapy Research*, 8: 399-402, 1994 ; Da-Silva et al, *Acta Tropica*, 60: 201-210, 1995) and nitric oxide production (Da-Silva et al, *Parasitology* 118: 575-582, 1999.). In the present work we investigated whether Kp treatment can modulate production of cytokines in normal and infected mice. We also investigated the effect of palmitic acid, a major fatty acid present in the most immunossupressive fraction of Kp, on cytokine production by normal mice.

Normal mice (4-5/group) were orally treated with 8mg of Kp extract or palmitic acid for 3 consecutive days, and then sacrificed. Alternatively mice (5-6/group) were infected in the footpad with 2×10^6 *L. amazonensis* promastigotes and from day 7 on daily treated with Kp extract. Lesion sizes were measured twice a week. Analysis of cytokine production by Con A-stimulated splenn cells was performed by ELISA (IFN-γ, IL-4 and IL-10) or bioassay (IL-2, CTLL).

The results indicated that oral treatment of normal mice with Kp extract induced an increased of IL-2 (15%) and IFN-γ (80 %) , but did not modify IL-4 and IL-10 production in relation to untreated mice. Palmitic acid treatment strongly increased IFN-g (190 %) but did not modify significantly the other cytokines. As to infected mice, we observed increase in IL-2 (150%) but no change in IFN-γ, Il-4 or IL-10 production on day 80 of infection. On day 110, Kp treatment produced increase in IFN-γ (81%) and a decrease IL-4 (40%) in relation to untreated controls. Lesion sizes were totally controlled in the Kp treated mice by day 50 of infection.

These results indicate that besides the induction of nitric oxide, the protective effect of oral Kp in leishmaniasis may involve a selective suppression of Th2-type response favouring Th1 development. The observation that palmitic acid present in an apolar fraction also up-regulate Th1 responses indicate that it may be important in the protective activity of Kp.

QM-3 – *IN VITRO* STUDIES WITH A NEW CLASS OF COMPOUNDS ACTIVE ON *TRYPANOSOMA CRUZI*: ALKYL-LYSOPHOSPHOLIPIDS

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Alkyl-lysophospholipids (ALPs) are under clinical investigations for cancer chemotherapy. This fact ensures data on the pharmacology and toxicology in humans, reducing the costs of developing potential drugs against tropical parasitic diseases. Edelfosine, ilmofosine and mitelfosine presented lytic activity against the three forms of *Trypanosoma cruzi*. In different experimental conditions, higher activity of the compounds against bloodstream trypomastigotes (8-16X) was observed in comparison with crystal violet. The values of ED_{50}/24h, under edelfosine treatment, were 13.4 ± 2.8 μM and 11.7 ± 0.6 μM for amastigotes and epimastigotes, respectively. Protection to the ALPs cytotoxic effect was observed in the presence of fetal calf serum. Ultrastructural analysis showed that ALPs caused several damages to the parasite plasma membrane as already described for tumour cells. Other alterations observed in the parasite were at the level of the mitochondria and of lipid inclusions, as well as progressive vacuolization of the cytoplasm. Edelfosine (0.3 and 0.6 μM) inhibited in 40-57% the metacyclogenesis process of parasites of Dm28C clone. This drug (3.75 to 15 μM) also caused a time- and dose-dependent inhibition of the *T. cruzi* infection in heart muscle cells, while the intracellular differentiation to trypomastigotes was not hampered by the drug. Pre-treatment of the parasites also inhibited their interiorization into the host cells.

Treatment with edelfosine led to an increase in calcium levels in amastigote and trypomastigote forms, while no effect was observed in epimastigotes. However, our experimental conditions could not discriminate whether this effect was due to a mobilization of intracellular stores or to a calcium entry from the extracelular medium. This ALP also caused a dose-dependent inhibition of phosphatidylcholine synthesis in epimastigotes, that occurred before the antiproliferative effect and could be associated with inhibition of the Greenberg route. It was observed a reduction in cholesterol uptake and an inhibition of ergosterol synthesis at the level of sterol 22-desaturase involved in the desaturation of 24-methyl-5,7,22-colesta, leading to accumulation of 24-ethyl-5,7-colesta-dien-3β-ol. The present results show that ALPs inhibit both proliferation and differentiation of *T. cruzi* as well as the infection in heart muscle cell. Preliminary results point out to the involvment of inhibition of lipid synthesis in their mechanism of action.

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QM-4 – ALKYLANTING COMPOUNDS AS ALTERNATIVE DRUGS TO EXPERIMENTAL ACUTE CHAGAS' DISEASE TREATMENT

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Drugs currently available for Chagas' disease treatment diminish symptomatic acute phase, but no side effects and resistance to treatment. Thus, it is important the assay of alternative drugs to treat acute phase of Trypanosoma cruzi infection. Chlorambucil 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, trypomastigote and amastigote forms of T. cruzi were incubated with CLB. The largest trypanocidal effect have been observed with CLB 304.2 mg/ml, that reduced significantly the growth and viability of epimastigote culture forms, and also reduced viability of amastigote and 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 CLB 3.0 mg/kg, 0.3 mg/kg, 0.03 mg/kg and 0.003 mg/kg, administered ip. in alternated days until death of all animals of the group. In B10.A mice we have increasing levels of parasitaemia until day 8 after infection, in treated and control groups. After 10 days of infection, animals treated with CLB 3.0 mg/Kg 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 of treated groups, and mortality started 10 days after infection and was significantly different of the mortality ratio showed in untreated controls. To evaluated the effect of CLB administered orally, B10.A mice were infected (10^3 trypomastigote, Y strain, ip) and treated with CLB 1.5 mg/ Kg in alternated days until death of all animals of the group. In this group the animals have increasing levels of parasitaemia until day 7 after infection and treated mice showed clearly smaller levels of parasitaemia than untreated control mice. Mortality started at day 11 after infection and was not significantly different between treated and untreated animals. ELISA was performed to determine levels of anti- T. cruzi antibody.

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QM-5 – FOLLOW-UP OF CHAGAS DISEASE TREATMENT WITH BENZNIDAZOLE BY SEROLOGY

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Treatment of non-acute Chagas disease is still controversial. The difficulty to demonstrate the parasite, associated with the lack of a gold standard laboratory method and/or a clinical parameter which would assure the presence or absence of the parasite, have hampered the evaluation of the efficacy of therapeutical agents, not forgetting the need of a very long-term follow-up. One option to evaluate the efficacy of cure in many infectious diseases is the search of serum specific antibodies, which tend to decrease or disappear after cure. This approach has been tried out in chagasic patients with conflicting results. The purpose of this work was to evaluate the serological evolution of chagasic patients after treatment with 200 mg/kg/day for 60 days with benznidazole. A total of 48 patients (27 indeterminate and 21 with cardiac chagasic dysfunctions, 66% and 52% with 6 to 12 years of follow-up, respectively) were examined before and after treatment (average 6.9 ± 3 years). The presence of specific antibodies were searched through an ELISA test, using total epimastigote *T. cruzi* antigen, Y strain. All sera were tested in parallel, using the same batch of antigen and buffers. Sera was serially diluted starting 1:40, and patients were divided in three subgroups according to the levels of antibody titers: negative, low (1:40 to 1:160) and high (≥1:320). The majority of the patients presented higher titers before therapy: 78% of those with indeterminate form and 71% of the cardiac patients. Only 18% of the total patients showed a decrease to low levels of antibodies, mainly within the indeterminate group (33% versus 9,5% of the cardiac group, $p < 0.0001$). None of the sera were negative after therapy, and three of them (6.2%) showed titers of 1:40. The levels were maintained high in 67% of the indeterminate group and 87% of the cardiac patients. In the latter, only 13% decreased to low levels. These results demonstrate that none of the patients fulfilled the criteria of complete parasitological cure. Alternatively, if the therapy was efficacious, serology is not indicated to follow-up the success of therapy for *T. cruzi* infection.

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QM-6 – IMPACT OF THE GENETIC DIVERSITY OF *TRYPANOSOMA CRUZI* ON ITS SUSCEPTIBILITY TO BENZNIDAZOLE *IN VIVO*

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Fifteen stocks pertained to the 19/20 (8 stocks) , 39 (3 stocks) and 32 (4 stocks) *T. cruzi* distinct genetic groups (Tibayrenc and Ayala, 1988) were assayed. Groups of 20 Balb/c mice were inoculated with 10^4 blood trypomastigotes of each stock. Ten of these mice were treated with Benznidazole 100mg/kg/day during 20 consecutive days as follow: 5 mice 10 days after inoculation (acute phase - AP) and 5 mice 90 days after inoculation (chronic phase - CP). The other 10 mice were maintained as control not treated. All mice were examined 30 days after treatment to verify the presence of the parasite by hemoculture and polimerase chain reaction (PCR). Sera were also collected 3 and 6 months later to ELISA and Flow Citometry (FACScan) to detect anti-live trypomastigotes antibodies (ALTA). Percentages of negative tests are showed as follow:

Genetic group →		19/20		39		32	
	Phase of	Percentage of negative tests					
Test	infection	Treated	Not treated	Treated	Not treated	Treated	Not treated
Hemoculture	AP	26,92	7,30	33,33	16,66	73,68	11,11
	CP	40,00	11,11	73,33	70,00	86,66	0,00
ELISA	AP	13,16	0,00	18,19	0.00	89,48	35,71
	CP	4,00	0,00	16,67	0,00	39,00	10,00
FACScan(ALTA)	AP	16,00	0,00	23,08	0,00	88,89	20,00
	CP	4,55	0,00	16,67	0,00	55,55	10,00
% of cure	AP	25,00	-	14,28	-	70,59	-
	CP	13,33	-	30,77	-	86,67	-

AP = Acute phase; CP = Chronic phase

The percentages of negative hemoculture, ELISA and FACScan (ALTA) tests founded in the AP were higher in mice infected with *T.cruzi* 32 genotype group and significantly different from those infected with 39 and 19/20 genotypes (32 > and # 39 >19/20). Similar results were observed in the CP (32 > 39 > 19/20) but in this case 32 # 39 # 19/20 except for hemoculture coincidently considered a poor method to detect parasites during the CP. Considering the 3 tests used the percentage of cure observed after AP treatment was 32 > and # 19/20 > 39). However in mice treated during the CP the rates of cure were 32 > and # 39 > and # 19/20. Preliminary results of PCR confirmed the other tests.

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QM-7 – *IN VITRO* AND *IN VIVO* EVALUATION OF TRYPANOSOMICID ACTIVITY OF MEGAZOL ANALOGS

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Chagas disease, caused by *Trypanosoma cruzi*, is an important endemic illness in Latin America, where an estimated 16-18 million persons are chronically infected and approximately 50.000 patients die each year because of the disease. Chemotherapy of Chagas disease is limited to the drugs benznidazole and nifurtimox, each of which presents low efficacy and several side effects. Megazol, [2-amino-5(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole] is a broad spectrum antibacterial and antiparasitic compound, but has mutagenic activity. We have prepared several analogs of Megazol, changing the substituents at the position 1 of the imidazole ring and the position 2 at the thiadiazole ring: Mega-S - [2-amino-5(1-ethylthioethyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole]. 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] e Mega-Br - [2-bromo-5(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole]. These analogs were evaluated towards the antiparasitic capacity in the *in vitro* culture using the litic assay of trypomastigotes forms obtained from mammalian cell culture. All compounds were 100% litic in 24 hours of incubation at 37°C. The minimal concentration displaying trypanolitic activity was established as follows: Megazol (10mg/mL), Mega-S (100 mg/mL), Mega-Br and Mega-T (300 mg/mL) and Mega-G and Mega-A (1mg/mL). Balb/c mice, i.p. infected with 10^4 blood trypomastigotes forms of Y strain of *T. cruzi* , were treated with the different compounds at 100 mg/kg administrated orally, starting at the time of infection. The results demonstrated that only Mega-Br reduces the parasitemia compared with untreated controls. Taken together, these results suggest that Mega-Br might be a promising drug to the treatment of Chagas disease.

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QM-8 – NEW MEGAZOL DERIVATIVES: SYNTHESIS AND ACTIVITIES AGAINST *TRYPANOSOMA CRUZI*

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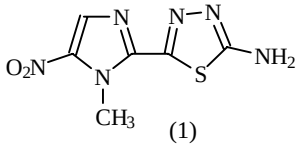
The *T. cruzi* life cycle of involves multiple developmental stages in both the insect vector and the mammalian host. *T. cruzi* is found transiently as a nonreplicative bloodsteam trypomastigote which invades cells and differentiates into replicative intracellular amastigotes. Epimastigote and metacyclic trypomastigote forms are found the insect vector. Since no efficient drugs for the treatment of chronically infected patients are available, new compounds should be seeked involving steps in the parasite's metabolic pathway which are absent or different from the host. Newer, less toxic chemical agents suitable for Chagas' disease chemotherapy are still urgently needed since only Nifurtimox and Benznidazol, both highly toxic, are currently clinically employed. This work presents results which were obtained in an attempt to identify a new class of nitroimidazole derivatives which might be effective against *T. cruzi* infections. The base structure of this class is Megazol [2-amino-5(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole] (1).



(1)

This compound was synthesized in 1968 by Asato and Berkelhammer and proved to be a broad spectrum antibacterial and anti-parasitic compound. This drug has also shown a marked curative effect in experimental Chagas' disease, being effective against strains that are resistant to both Nifurtimox and Benznidazole. We have been working with of Megazol derivatives, studying their activity against *T. cruzi*. In preliminary tests using Vero cells infected with *T. cruzi* amastigotes and also axenically grown epimastigotes, we have demonstrated that Megazol (1mM) and their acetylated derivative (1μM) have greater activity than Benznidazol (10μM).

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QM-9 – EVALUATION OF PURINE NUCLEOTIDE ANALOGUE ACTIVITY AGAINST *TRYPANOSOMA CRUZI* EPIMASTIGOTES *IN VITRO*

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Nifurtimox and Benznidazol are the most utilized drugs in the treatment of Chagas' disease, but they are incapable of erradicating the parasite. There are many toxic side effects presented by Nifurtimox (Levi, *et al*, 1975, *Rev. Inst. Med. Trop. São Paulo*, 17:49-54; Barclay, 1977, *Rev. Neurol*., Arg., 3:477-482; Cançado, 1985, *Cardiopatia chagásica*, p.327-55) and Benznidazol was not efficient in transplanted patients and still allows a high rate of recurrence (Bocchi, 1993_b – Tese de Doutorado – FMUSP). For the treatment of Chagas' disease, alternative drugs are necessary with more parasiticide power and less incidence of toxic side effects (Marr & Docampo, 1986, *Reviews Inf. Dis*., 8:884-903; Castro, 1993, *Acta Trop*., 53:83-9). The purine analogues are of great interest, mainly the Allopurinol used in gout treatment, inhibiting the action of xanthine oxidase, thus reducing the oxidation of hypoxanthine to xanthine and xanthine to uric acid. Allopurinol also belongs to the class of nucleotide biosynthesis inhibitor from the purine salvage way. The knowledge of the molecular mechanism envolved in the purine salvage, which is fundamental to the protozoan's survival, can be explored to produce new chemotherapeutic strategies (Marr, 1991, *J. Lab. Clin. Med.*, 118:111-9). Marr & Berens, 1978, *Science*, 201:1018-20, studying a tissue culture system, demonstrated that the allopurinol is capable of killing *T.cruzi*, suggesting that this compound can erradicate the infection even without an immune response present. The evaluation the possible effect of this compound against *T.cruzi* epimastigotes in LIT medium was the aim of this study.

Epimastigotes, at a concentration of 5.10^5 cells/ml were grown in a LIT medium, at 28^o C, in the presence or absence of the allopurinol (40 μg/ml). Cell counting was performed using a Neubauer chamber daily from the 3rd day until the 7th day. After this, it was counted every three days until the 40th day. Important differences were observed in the epimastigotes of the control group (without Allopurinol) and the experimental group (with Allopurinol) from the 6th day until the 40th day. The whole time the control group had more epimastigotes forms than the experimental group. However, the most important differences in epimastigotes' number was verified on the 14th and 18th days, with 6.10^8/ml and $6.4.10^8$/ml in the control group, and $0.8.10^8$/ml and $0.6.10^8$/ml in the experimental group, respectively. Some anti-parasitic action of Allopurinol was shown in this preliminary analysis.

QM-10 – EFFECT OF MICROTUBULES INHIBITORS ON *TRYPANOSOMA CRUZI*

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In the present work, we investigated the effect of taxoids, *Vinca* alkaloids and dinitroanilines as microtubules inhibitors on *T. cruzi* (Bell, Parasitol Res 79:146, 1998). The approach of targeting the microtubules was a successful one in cancer and antihelmintic chemotherapies. Taxol (**Tx**), an anticancer drug that binds to microtubules, showed activity against *T. gondii* (Estes et al., *Antimicrob Agents Chemother* 42:2036,1998) and African trypanosomes (Kaminsky & Zweygarth, *Antimicrob Agents Chemother* 33:881,1989). Vincristine (**Vc**) is an alkaloid from *Catharantus roseus* (Himes, *Pharmacol Ther* 51:257-267,1991), that is used extensively in cancer chemotherapy, in spite of its toxicity. The dinitroaniline trifluralin (**Tf**) is a microtubule-disrupting herbicide shown to be active against different pathogenic protozoa. It was claimed that **Tf** leishmanicidal activity (Chan et al., Antimicrob Agents Chemother 37:1909, 1993) was associated with the presence of chloralin (**Cl**) (4-chloro-3,5-dinitrobenzotrifluoride), a contaminant of its synthesis (Callahan et al., Antimicrob Agents Chemother 40:947, 1996). Since **Tf** binds to plant but not animal tubulins, it is a promising lead compounds for antiparasitic agents.

Previous work of our group showed the activity of **Tf** and **Cl** against epimastigotes of *T. cruzi* (Y strain and clone DM28c), being 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 (Dantas et al., *Mem Inst Oswaldo Cruz* 93 (suppl.II):311, 1998). It was also observed an apparent relationship between susceptibility to **Tf** and the β-tubulin sequence of a given organism (Ortigão et al., *Mem Inst Oswaldo Cruz* 92 (suppl.):24, 1997). Extending this study to other microtubule inhibitors, we analysed the effect of **Tx** and **Vc** on *T. cruzi*. Against trypomastigotes **Tx** presented activity about 1000 times higher than **Tf** with ED_{50}/24h = 0.114±0.043 and 129.5±8.9μM, respectively. The effect of **Vc** and **Cl** were similar (ED_{50}/24h =56.1±2.5 and 49.2±1.7μM) about 3x higher than **Tf** and about 500x lower than **Tx**.

The treatment with **Tf** caused in epimastigotes intense vacuolization of the cytoplasm and swelling of the mitochondria with loss of the inner membrane organization. In trypomastigotes, the effect was on the plasma membrane, with formation of blebs. With both forms of the parasite, by routine electron microscopy technique, no damage was observed on subpellicular microtubules or para-axial structure. On the other hand, the effect of **Cl** on epimastigotes was localized on the mitochondria, with alterations on the kDNA network. Experiments are underway to analyse the alterations caused by the drugs at the cytoskeleton level.

Supported by PAPES/FIOCRUZ and CNPq

QM-11 – ACTIVITY OF AROMATIC GUANYLHYDRAZONES *IN VITRO* AND *IN VIVO* AGAINST BLOOD TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI*

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Guanylhydrazones represent a class of drugs which have shown trypanocidal activity. Over the past decad, several hundred guanylhydrazones derivatives have been synthesized and their structure-activity relation ships have been studied in mouse model of African trypanosomiasis. In a pioneer study, we found that these compounds were potent growth inhibitors of Trypanosoma cruzi tripomastigotes (Messeder et al., *Bioorg. & Med. Chem. Lett.*, Vol.5, 24:3079-3084). We have prepared a second generation of guanylhydrazones and studied their effect on *T. cruzi* tripomastigotes with the result from the first generation of these drugs. Chemical modifications were done in the strucuture of the basic molecule, in order to increase the activity and we found that the most actives compounds were the methoxilated derivatives. In this work we are now showing our *in vivo* result with these drugs. A total of 48 compounds screened in this assay. The aromatic guanylhydrazones were prepared by direct reaction of the respective aldehydes with aminoguanidine hydrochloride in refluxing dry toluene with p-toluene sulfonic acid as catalyst. To determine *in vitro* activity, controls containing 2,5% DMSO or gentian violet at its IC 50 (18µ M) were run in parallel. After 24h at 4º 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 field 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 the parasite survival. We found that compound di-methoxilated (2,3-di-methoxibenzaldehyde guanylhydrazone hydrochloride) was highly effective at clearing parasites from infected blood (IC50 = 22,5 µM). According to *in vitro* results, we decided study the possible action of most active aromatic guanylhydrazones in reducing parasitaemia and mortality ratio of *T.cruzi* infected mice. In the *in vivo* experiments groups of 15 mice were used which were inoculated with 5 x 10^4 parasites. The drugs were administred in a concentration of 100mg/Kg/weigth, for 7 days (orally treatment). The results showed that the substituition in the benzene ring of aromatic guanylhydrazones with halogen and methoxi groups ted to lytic activity against blood trypomastigotes of *T. cruzi*. In the *in vivo* experiments several groups mice treated with di-methoxilated coumpound showed reduction of the parasite number in 92%. Although the mecanism of trypanocidal action of these compounds is not known, interation with biomembranes is a likely key factor in the activity of these cationic compounds against *Tripanosoma cruzi*. Therefore, the compounds pathway may represent a different approach for the development of new antitrypanosoma drugs. Studies to address these questions are currently in progress in our laboratory.

Supported by Capes.

QM-12 – A MOLECULAR MODELING COMPARATIVE STUDY BETWEEN AMINO GUANYL AMIDES AND GUANYL HYDRAZONES LIKE TRYPANOCIDE AGENTS USING EMPIRICAL METHO

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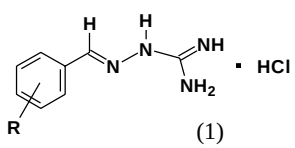
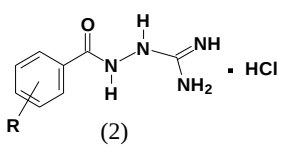
The monocationic guanyl hydrazones (1) have been studied as trypanocides substances, showing *in vitro* and *in vivo* activity against *Trypanosoma cruzi*. Another class of monocationic substances containing a guanidine group, the aminoguanyl amides (2), which may present a shorter half life and lower toxicity, were designed in our group. The presence of an amide moiety in the aminoguanyl amides suggests taht these drugs may be more metabolizable than guanyl hydrazones. A comparative study of both types of compounds was carried out using molecular mechanics as well as some *in vitro* activity results. In this comparison a CVFF and AMBER were utilized like forcefields, and a docking methodology was used too study the intermolecular energy of the interaction drug-DNA. A previously minimized B-DNA dodecamer was used a model for B-DNA in the docking studies with the chosen aminoguanyl amides and guanyl hydrazones. The conformational analysis was carried out for 5 aminoguanyl amides and the equivalent 5 guanyl hydrazones using Torsion Drive methodology, with the angles changing for about 18º. For the bioassays, trypomastigote forms of the parasite were obtained from mices inoculated intraperitoneally with 10^5 cells of Y strain *T. cruzi*. , and the values of IC_{50} were obtained by linear and polynomial regression analysis.

The results show that the degree of planarity of the molecule is related with lower docking energies, with the aminoguanyl amides being more planar than the guanyl hydrazones. This greater stability of the aminoguanyl amide-DNA complex is followed by lower values of IC_{50} indicating that the drug-DNA interaction may play a role in the mechanism of action of these compounds. For example, the docking energy for the aminoguanyl amide of benzaldehyde is 4 kcal/mol lower than the enregy for the respective guanyl hydrazone (205 µM) and is also more active (ID_{50} 175 µM).



(1)



(2)

Financial suport: PADCT/CNPq, FAPERJ, CAPES.

QM-13 – TRYPANOSOMICIDAL ACTIVITY OF *ECHINODORUS GRANDIFLORUS* (CHAMISSO & SCHLECHTENDAL) MICHELI.

Stutz, C..M. (2); Soares, R.O.A.(2); Fernandez-Ferreira, E.(2); Pimenta, D.S.(1); Gibaldi, D.(2); Bozza, M.(2); Figueiredo, M.R.(1) & Kaplan, M.A.C.(3);
1-Laboratório de Química de Produtos Naturais, PN3; 2- Laboratório de Farmacologia Aplicada 1, Far-Manguinhos/ FIOCRUZ e 3-Núcleo de Pesquisa de Produtos Naturais/UFRJ

Echinodorus grandiflorus known "chapéu de couro" is used in folk medicine to treat several diseases. Crude hexane, alcohol (methanol, butanol) and aqueous extracts as well as the ethyl acetate and the aqueous fractions of methanol extract partition from fresh and dry leaves and rhizomes from *E. grandiflorus* have been tested for trypanosomicidal activity. The extracts were diluted in RPMI medium and used in the concentration of 250 $\mu g/5x10^5$ trypomastigote forms of the parasite *Trypanosoma cruzi* of Y strain, obtained from LLC-MK2 culture. The percentage mortality was determined after 24h and 48h incubation, by comparison with the negative and positive controls (culture medium and violet crystal, respectively). The tested samples showed 100% activity exception for the hexane and the aqueous fraction of methanol extract partition that showed negative results. These assays revealed *E. grandiflorus* crude extracts as a good trypanosomicidal agent.

QM-14 – TRYPANOSOMICIDAL ACTIVITY OF *QUESNELIA QUESNELIANA* (BRONGNIART) L.B.SMITH (BROMELIACEAE)

Gibaldi, D.(2); Chedier, L.M.(1); Soares, R.O.A.(2); Fernandez-Ferreira, E.(2); Stutz, C..M. (2); Bozza, M.(2); Figueiredo, M.R.(1) & Kaplan, M.A.C.(3);
1-Laboratório de Química de Produtos Naturais, PN3; 2- Laboratório de Farmacologia Aplicada 1, Far-Manguinhos/ FIOCRUZ e 3-Núcleo de Pesquisa de Produtos Naturais/UFRJ.

Quesnelia quesneliana belongs to the family Bromeliaceae, order Bromeliales, superorder Liliiflorae (*sensu* Dahlgren, 1982). Some Bromeliaceae species have economical value as *Ananas comosus* (L.) Merril (pineaple), and some others are used in traditional medicine as anti-inflammatory, anti-helmintic, analgesic and diuretic. Among the secondary metabolites, flavonoids, terpenoids and steroids are the common representatives. *Quesnelia quesneliana* was collected at National Biological Reserve, Silva Jardim, RJ. Leaves, rhizomes and roots, after drying, were extracted successively with hexane and methanol yielding the hexane and methanol extracts. Aqueous extracts from these plant materials were obtained by infusion. The extracts were diluted in RPMI medium and used in the concentration of 250 $\mu g/5x10^5$ trypomastigote forms of the parasite *Trypanosoma cruzi*, obtained from LLC-MK2 culture. The percentage mortality was determined after 24h and 48h incubation, by comparison with the negative and positive controls (culture medium and violet crystal, respectively). All the crude extracts from *Q. quesneliana* showed *in vitro*, 100% activity after 24h incubation revealing thus a good trypanosomicidal activity on trypomastigotes forms of *Trypanosoma cruzi*.

QM-15 – TRYPANOSOMICIDAL ACTIVITY OF *NIDULARIUM INNOCENTII* LEMAIRE (BROMELIACEAE)

Soares, R.O.A.(2); Chedier, L.M.(1); Fernandez-Ferreira, E.(2); Gibaldi, D.(2); Stutz, C..M. (2); Bozza, M.(2); Figueiredo, M.R.(1) & Kaplan, M.A.C.(3);
1-Laboratório de Química de Produtos Naturais, PN3; 2- Laboratório de Farmacologia Aplicada 1, Far-Manguinhos/ FIOCRUZ e 3-Núcleo de Pesquisa de Produtos Naturais/UFRJ.

Bromeliaceae is a family belonging to the superorder Liliiflorae, order Bromeliales (*sensu* Dahlgren, 1982). This family consists of 50 genera and about 2500 species, many of which are used in popular medicine as anti-inflammatory, analgesic, anti-helmintic and diuretic. *Nidularium innocentii* Lemaire was collected at Poço das Antas National Park, Silva Jardim, RJ. Leaves (970g) and rhizomes/roots (670g) of *N. innocentii* were ground and submitted in sequence to extraction with hexane and methanol. Methanol extract of rhizomes/roots suspended in water were submitted to liquid-liquid partitions with hexane, dichloromethane, ethyl acetate and butanol. The aqueous extracts of leaves and rhizomes/roots were obtained by infusion. All the extracts and fractions from *N. innocentti* were tested for the trypanosomicidal activity. The plant material extracts and fractions were diluted in RPMI medium and used in the concentration of 250 $\mu g/5x10^5$ trypomastigote forms of the parasite *Trypanosoma cruzi* obtained from LLC-MK2 culture. The percentage mortality was determined after 24h and 48h incubation, by comparison with the negative and positive controls (culture medium and violet crystal, respectively). Leaf extracts presented activities over 90% after 24h incubation. The rhizomes/roots crude hexanic extract showed to be negative. The methanol and aqueous extracts of rhizomes/roots presented 100% and 90% activities, respectively. The ethyl acetate fraction from methanol extract partition showed 100% activity after 24h incubation. The hexane fraction showed to be negative, while the dichloromethane and the butanol fractions were actives after 48h incubation. These results revealed the *N. innocentii* leaves and rhizomes/roots extracts to have good trypanosomicidal activities.

QM-16 – CHEMICAL REACTIVITY STUDIES WITH NAPHTHOQUINONES FROM *TABEBUIA* AS A GUIDANCE TO THE SEARCH FOR TRYPANOCIDAL DRUGS

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The biological activities of the naphthoquinones lapachol and its heterocyclic derivative b-lapachone, extracted from trees of the genus *Tabebuia*, have been intensively studied. The diversity of microbicidal effects, the easy access to natural sources of these quinones in Brazilian rain forest, and the synthetic alternative routes already developed by our group for the electrophylic reactivity of quinoidal carbonyl towards nucleophylic agents led us to consider lapachol and β-lapachone as starting points for medicinal chemistry studies. Previous work of our group showed trypanocidal activity of some new heterocyclic derivatives obtained from the reaction of those naphtoquinones with amino-containing reagents (Pinto et al., *Arzneim-Forsch 47 (I)*:74, 1997).

Given continuity to our studies, we synthesised 21 derivatives from β-lapachone, nor-b-lapachone and lapachol and analysed their trypanocidal activities against trypomastigote forms of *T. cruzi* (Y strain). The compounds were grouped as oxazolic, imidazolic, oxazinic, pyrrolidinic, pyranic and cyclopentenic derivatives. The variability of the new structures comes from the great electrophylicity of 1,2-quinoidal carbonyls towards reagents containing nitrogen or cabon as nucleophilic centers. In relation to trypanocidal activity of the synthesized compounds, due to the structural diversity of the assayed compounds, we could only analyse tendencies. Among the cyclofunctionalizations developed both oxazolic and imidazolic derivatives showed higher activity, in the range of 1.5 to 34.8 times in relation to crystal violet, the standard drug.

The presence of imidazolic or oxozolic moieties increasing the trypanocidal activity might be due to structural factors, specially in the case of imidazolic derivatives. It is important to note that several substances described as trypanocidal agents, contains a basic indolic (Leon et al., *Exp Parasitol 45*:151, 1978, Haun et al., *Biol Res 25*:21,1992, Sepúlveda-Boza & Cassels, *Planta Med 62*:98, 1996) and imidazolic (Winkelmann et al., *Arzneim-Forsch 28*:351,1978, McCabe et al., *Am J Trop Med Hyg 32*:960,1983, Urbina et al., *Mol Biochem Parasitol 30*: 185,1988, Chabala et al., *Experientia 47:*51,1991, Nothenberg et al., *J Inorg Biochem 42*:217,1991, Blandon et al., *Rev Med Panama 18*:94,1993) moieties, including benznidazole used for the treatment of Chagas disease. Our results corroborates the tendency of trypanocidal activity in imidazolic skeletons, and indicates that this moiety in an architectural delineation of molecules of potential value for the chemotherapy of Chagas disease. As a working hypothesis, it is possible that quinoidal drugs display their biological activity due to interaction with nucleophylic centers of enzymes in fundamental metabolic processes, leading to their inactivation. This type of interaction could be irreversible, due to the formation of heterocyclic structures as shown by our present results, ando also could be associated with the precipitation of blood components by b-lapachone as previously described (Gonçalves et al., *Mol Biochem Parasitol 1*:167, 1980).

Supported by PAPES/FIOCRUZ and CNPq

QM-17 – EFFECT OF SYNTHETIC PEPTIDES CECROPIN/MELLITIN ON *LEISHMANIA AMAZONENSIS* PROMASTIGOTE CELLS

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Antibiotic peptides have been found in animal (vertebrate and invertebrate) and plants kingdom but most of the reports have focused on bacterial and fungi than eukaryotic cells. They have a considerable structural diversity and consequent amino acid heterogeneity. Many of them present a potential therapeutic application while others may be useful tools to probe and define important structural function of protein segments. In this work, flow cytometry, alkaline phosphatase activity and parasites mobility were used to study the effect of two commercials (cecropin A and mellitin) and two synthetic (I116 and II121) peptides on *Leishmania (L.) amazonensis* cells. For flow cytometry, *Leishmania* promastigote forms and *E. coli* (control) were grown for 4 hr with different concentrations of the synthetic peptides and after washing ethidium bromide was added and the optical density measured at 600 nm. *E. coli* but not *L. amazonensis* mixture cells containing 10 μg/10^6 cells of the peptide cecropin and mellitin presented 90% uptake of the dye. The uptake of the dye by *L. amazonensis* promastigote cells not incubated with the peptides or with identical concentration of cecropin peptide was lesser than 1%. These results with the increasing of the phophatase activity in the supernatant of cultures incubated with the cecropin and the two peptides demonstrated that the bacteria but not the *L. amazonensis* membrane is a target for lysis induced-pore formation. The analysis of other peptides is in progress.

Supported by FIOCRUZ and CNPq.

QM-18 – LEISHMANICIDAL EFFECT OF SYNTHETIC INDOLE ALKALOIDS

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Pentavalent antimonial compounds are still the first line treatment for leishmaniasis, a disease that affects around 3 million people world-wide. High costs, long-term treatment and side effects, associated with unresponsiveness to treatment, prompted us to search for new chemotherapeutic agents. We have previously shown a leishmanicidal effect of Coronaridine (an indole alkaloid) purified from *Peschiera australis* stem. To further investigate this activity we evaluated synthetic Coronaridine (hidrochloride salt) and 18-Metoxi-Coronaridine (racemic mixture and enatiomeric compounds) on *Leishmania amazonensis* amastigotes. *In vitro* infected mouse peritoneal macrophages were treated with the different drugs 24 hr after the infection and the parasite survival determined after 3 days. Synthetic coronaridine treatment induced a 60% decrease in the parasite survival, while natural coronaridine showed an inhibition of 43% at 10 μg/mL. The + 18-Metoxi-Coronaridine was also effective and 63% of the parasites were killed at the same concentration. The enatiomeric compounds were less effective. Glucantime, used as a control, inhibited 29% of the parasite growth. In order to further analyze the mechanism of the leishmanicidal effect, we measured nitric oxide (NO) prodution on J774.A1 macrophages by Griss assay. J774.A1 monolayers pre-activated or not with 100ng/mL lipopolysaccharide + 10% γ-IFN, were treated with the different compounds. Any of the tested compounds stimulated NO production. However, the drugs inhibited NO production induced by pre-activation.

Supported by: PRONEX, CNPq, UNDP/WORLD BANK/WHO-TDR.

QM-19 E QM-20 – NÃO ENVIADOS

QM-21 – COMPARISON BETWEEN CONTINUOUS AND INTERMITTENT SCHEDULES OF ANTIMONIAL THERAPY FOR CUTANEOUS LEISHMANIASIS IN THE MUNICIPALITY OF RIO DE JANEIRO.

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The antimonial treatment currently recommended by the Brazilian Ministry of Health for cutaneous leishmaniasis (CL) is: 10 to 20mg of Sb^v/Kg/day for 20 days, repeating this regimen if complete healing of lesions is not obtained within three months after its end. Another antimonial regimen widely used for treating cutaneous leishmaniasis in Brazil consisted of equivalent daily doses given in ten-day cycles alternated with ten-day rest intervals. The objective of this study was to compare these two therapeutic regimens with regard to adherence to therapy, proportion of therapeutic failure (need of further treatment), side effects and mean time period for healing of lesions. Patients from an area where CL is exclusively caused by *Leishmania braziliensis* were allocated into two groups with similar composition with regard to age, sex, number of lesions and period of disease progression before the onset of treatment. A group of 72 patients received the continuous schedule whereas the other group with 49 patients received the intermittent treatment. In both groups the daily doses of antimonial (Meglumina antimoniato, provided by the Brazilian Ministry of Health) were given by deep IM injections. All patients were evaluated weekly by the same medical personnel at outpatient units of the Health Secretariat of Rio de Janeiro´s Municipality. In the group receiving the continuous schedule, 19.4% of patients interrupted the therapy without medical consent, therapeutic failure occurred in 16.7% and side effects were observed in 23.9% of them. In the group that received the intermittent regimen, three patients abandoned the treatment (4.1%), there was 6.1% of therapeutic failure and side effects were observed in 16.3% of the subjects. The proportion of cured patients was significantly higher in the group taking the intermittent schedule ($P<0.05$, Fisher's exact test). A significantly higher adherence to the therapy was also observed in this group ($p<0.05$, Fisher's exact test). Considering only the patients who did not abandon the treatment, therapeutic failure was more frequent ($P<0.05$, Fisher's exact test) in the group receiving the continuous regimen (17.9%) than in the group taking intermittent therapy (6.4%). Nevertheless, considering only the patients who have cured, the mean time for the complete healing of lesions was similar between the groups: 107.5± 63.1 days for the continuous and 103.6 ± 53.9 days for the intermittent schedule. Our results show that the adherence to therapy and the cure rate provided by the intermittent schedule are higher than those obtained with the continuous schedule of antimonial therapy for CL in the Municipality of Rio de Janeiro.

Financial support: CNPq.

QM-22 – ACTION OF *POROPHYLLUM RUDERALE* (JACQ.) CASS. EXTRACT ON AMASTIGOTES FORMS OF *LEISHMANIA SP*. AND ON THE PRODUCTION OF NITRIC OXIDE

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The American Tegumentary Leishmaniasis (ATL) is an endemic disease and of high incidence in Brazil. Countless plants are used popularly in the treatment of ATL, among them, the "cravinho", *Porophyllum ruderale* (Jacq.) Cass. In this work we evaluated the effect of this vegetable species on amastigotes forms of *Leishmania "in vitro"* and in the treatment of infected mice experimentally. In the *"in vitro"* experiments, peritoneal macrophages (M∅) of BALB/c mice, elicited with thioglycollate, were cultivated on glass coverslips, in plates of 24 wells containing RPMI+10% of FCS, soon after infected with promastigotes forms of *Leishmania (Leishmania) amazonensis* and treated with different concentrations of the crude extract. After 24 hours at 37° C and 5% of CO_2 the coverslips were stained with Giemsa. The infection index (percentage of infected macrophages x average number of amastigotes per macrophage) was determined by count of at least 200 cells in common optical microscope. The results showed that in the concentrations of 1,5 and 1,0 mg/ml the crude extract of *P. ruderale* inhibited in 55,8% and 37,2%, respectively, the multiplication of the amastigotes forms of *L. (L.) amazonensis*.

It was also investigated the effect of the crude extract of *P. ruderale* on the production of nitric oxide (NO) by peritonial M∅ elicited from BALB/c mice, after stimulated with INF-γ (50UI/ml) and LPS(50 ng/ml). After 48 hours of cultivation the supernatant was removed and the quantified nitrites levels with the reagent of Greiss. The crude extract inhibited the production of NO for macrophages stimulated with INF-γ/LPS in 91,7%, 70,7% and 43,8%, in the concentrations of 1,5, 1,0 and 0,5 mg/ml, respectively. This inhibition can imply so much in the inactivation of key enzymes in the synthesis of NO, as the nitric oxide-synthase (iNOS), as in the regulation of other mediators that modulate in macrophages the production of NO. The activity of the crude extract of *P. ruderale* was evaluated in three groups of 9 mice. Two groups were infected in the left posterior paw with $1x10^7$ promastigotes, having one of them received treatment orally with the lyophilized crude extract in the dose of 200 mg/kg of weight. The third group, not infected, received just the treatment. The effect of the treatment on the evolution of the infection was evaluated by the weekly measure of the thickening of the paw. There was no significant difference statistically between the treated and the non treated groups. The pathway of administration chosen may not have been adequate or the concentration of the active principle in the crude extract not to have been enough to reach the medicinal effect.

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QM-23 – IN VIVO SENSITIVITY OF *PLASMODIUM VIVAX* ISOLATES FROM RONDONIA (WESTERN AMAZON REGION, BRAZIL) TO CHLOROQUINE AND TO CHLOROQUINE-PRIMAQUINE ASSOCIATION

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Seventy nine adult patients with vivax malaria infectons from the Porto Velho area of Rondonia gave their informed consent to participate in a double blind clinical assay to compare the efficiency of two therapeutic schedules combing chloroquine and primaquine: a classical one, recommended by WHO and Fundação Nacional de Saúde (SUCEN, 1997) , with administration of chloroquine for 3 days and primaquine for 14 days; a second alternative "short" schedule combining chloroquine and primaquine simultaneously for 5 days. Samples of medicine tablets provided to patients were analyzed in the Far-Manguinhos Institute (FIOCRUZ) for precise dosage of the active principles. The schedule used for drug administration, included personal surveillance for guarantee ingestion of medicine tablets and absence of vomiting. Microscopic examination of Giemsa stained thick and thin blood smears from the patients were performed daily until clearence of blood parasites, weekly in the first month following treatment and then once a month in the second and third months. From the initial 79 patients, 73 had complete follow up for the first 30 days with no cases of recrudescence indicative of chloroquine resistance.

Ten cases of relapses were observed, all after 60 days following treatment, from which eight belonged to the group of patients that received the "short" treatment schedule and two that received the classical treatment. Performed PCR amplification with DNA from the original and relapse parasites to characterize the corresponding MSP-1 allele suggest that two populations from the putative relapses corresponded in fact to re-infections. The results indicate that the classic schedule of association of chloroquine and primaquine is more effective to avoid relapses. However, considering the side effects of prolonged primaquine administration that frequently provoke the abandon by the patient treated at home without surveillance, the "short" schedule could be a useful alternative in malaria control to avoid the current high frequency of relapses observed in endemic areas of the Amazon region.

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QM-24 – EVALUATION OF A MACROLIDE ANTIBIOTIC IN MICE INFECTED BY *PLASMODIUM BERGHEI*

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According to Kuschner *et al*., 1994, *Lancet*, (343): 1396-1397, azithromycin, an azalide antibiotic related to macrolides, has antimalarial activity and favorable pharmacokinetic properties for a prophylatic antimalarial agent. Inhibition of parasite mitochondrial protein synthesis, a suggested mechanism of action for tetracycline, is probably the mechanism by which azithromycin acts as antimalarial. With the intention of increasing the number of drugs utilized in the malaria treatment, the action of azithromycin in mice infected by *Plasmodium berghei* was evaluated in this study.

Forty BALB/c male mice were used, each one weighing aproximately 25 g. They were obtained from Biotério Central da FMUSP. They were separated in 4 groups: G1 – 10 were infected and treated with 10 mg/kg/day; G2 – 10 were infected and treated with 100 mg/kg/day; G3 – 10 were infected and not treated (infection control group); and G4 – 5 were treated with 10 mg/kg/day and the other 5 treated with 100 mg/kg/day (drug control group). Each mouse of groups G1, G2 and G3 was infected with 5.10^3 red cells infected with *Plasmodium berghei*. The azithromycin (Zitromax) was administrated daily, orally, in groups G1, G2 and G4 since the day of infection. The drug action was analyzed by mortality and parasitemia, on the 4^{th}, 7^{th}, 10^{th}, 14^{th}, 17^{th} e 23^{rd} days after infection, by Giemsa staining on the blood harvested from the mice's tail. Just in groups G1 and G3 the parasitemia was positive on 7^{th} day after the infection. In group G2 there was no parasitemia ocurrence. The mortality began among mice from group G3 on the 13^{rd}, and all of them died on the 21^{st} day after the infection; in group G1 it happened from the 18^{th} day onwards and by the 23^{th} day all of them had died; in the G2 and G4 there was no death. The antiparasitic action of the drug, in doses of 100 mg/kg/day, was proven by the parasitemia and survival preliminary analysis. Our study must continue to evaluate histopathologically dead animals.