Trypanocidal activity of novel alkanediamide-linked bisbenzamidines and bisbenzamidoximes

T. Huang1,∗, N. Kode2, C. Bacchi3, D. Rattendi3, J.J. Vandennye2, A. Mayence2, N. Yarlett1, I. Londono3

1 Xavier University of Louisiana, New Orleans, LA, USA
2 Xavier University of Louisiana, College of Pharmacy, New Orleans, LA, USA
3 Pace University, Haskins Laboratories, New York, NY, USA

Background: Human African trypansomiasis (HAT) is caused by the protozoan parasites Trypanosoma brucei gambiense (T.b.g) and Trypanosoma brucei rhodesiense (T.b.r) and is usually fatal when left untreated. It is one of the most neglected tropical diseases in the world causing an estimated 50,000 deaths annually. Current drug therapy suffers from high toxicity, undesirable intravenous route of administration and emergence of parasite resistance. The present study is to evaluate the trypanocidal activity of a novel series of alkanediamide-linked bisbenzamidines and bisbenzamidoximes against several clinical isolates of Trypanosoma brucei.

Methods: A series of 20 bisbenzamidines and bisbenzamidoximes were synthesized and tested in vitro against a drug-sensitive strain of T. b. brucei Lab 110 EATRO and a drugresistant strain of T. b. r. KETRI 243. The bisamidoximes were designed to improve oral bioavailability by functioning as orally-active produgs of the most active bisamidines. The in vivo efficacy of 8 bisbenzamidines and bisbenzamidoximes were evaluated using mice infected with the drug-sensitive (T. b. brucei Lab 110 EATRO) or drug-resistant strains of T.b.r. KETRI 2002 and KETRI 2538.

Results: The tested compounds generally showed similar in vitro potencies against both strains of T.b. The most potent compounds were bisbenzamidines linked with a hexanediamide, heptanediamide or octanediamide group (inhibitory concentration for 50% (IC50) = 1-3 nM). Several of the most potent bisbenzamidine compounds were effective in curing mice infected with the drug-sensitive or drug-resistant strains of T. b. rhodesiense. Curative doses were < 15 mg/kg/day for 3 days given by the intraperitoneal injection in the mouse model of infections. However, replacing the terminal basic bisamidines with less basic bisamidoxime groups resulted in prodrugs that were not orally effective against T. b. brucei infected mice.

Conclusion: The results suggest that alkanediamide-linked bisbenzamidines are highly effective against T. brucei, but further optimization of the prodrug strategy is needed to improve their oral bioavailability.

doi:10.1016/j.ijid.2010.02.594

A critical role of CD2 as an immunoprophylactic agent to combat visceral leishmaniasis

S. Sinha1,∗, S. Bimal2, S. Sundaram1

1 Allahabad University, Allahabad, India
2 Rajendra Memorial Research Institute for Medical Sciences, Patna, Bihar, India

Background: A major Concern for VL prevention appears to be the inability of their CD4+ T cells to mount an adequate TH1 response which ensures the possible cure of the disease. Similarly the effectiveness of SAG in intact animal is determined by the host cell-mediated immune response. The present study aims at evaluating the use of CD2 antibody as an immunotherapeutic agent along with SAG in ensuring treatment of BALB/c mice induced with experimental Visceral leishmaniasis.

Methods: Mice were infected with Leishmania donovani promastigotes. Another set served as control. After seven week of infection, a set of mice from infected group was subjected to SAG treatment and another group was subjected to SAG treatment along with stimulation with antiCD2 antibody. CD4 cells expressing CD-25 were immunophenotyped and cytokines like IL-2, IFN-γ and TNF-α were assessed using FACs. We also looked into cell cycle pattern, expression of CD25+ cells on T cells, percentage of lymphocytes converted into lymphoblasts, percentage of activated T lymphocytes and IL-2 production. These parameters were evaluated in T cells both before and after stimulation of their CD2 antigen.

Results: We recorded a substantial enhancement of protective cytokines which are essential for combating visceral leishmaniasis infection. We also observed a significant reduction in parasitic load when drugs are used in combination with this immunoprophylactic agent

Conclusion: CD2 proved to be an important immunoprophylactic agent which if used in combination with drugs can provide a suitable and substantial cure against visceral leishmaniasis.

doi:10.1016/j.ijid.2010.02.595

Patients with suspected visceral leishmaniasis in Istanbul

H. Cakan1,∗, S. Saribas2, V. Oz1, E. Polat3, M. Aslan2, B. Kocazeybek3

1 Istanbul University, Forensic Medicine Institute, Istanbul, Turkey
2 Istanbul University, Cerrahpasa Faculty of Medicine, Istanbul, Turkey
3 Istanbul University Cerrahpasa Faculty of Medicine, Turkey, Turkey

Background: Visceral Leishmaniasis (VL) is a parasitic disease caused by Leishmania infantum. It is transmitted through bites of infected sand flies (female Phlebotomus). We aimed to investigate bone marrow and blood samples obtained from the patients with suspected VL.

Methods: Fifty-nine patients with suspected VL from Istanbul were included in this work. Bone marrow and blood samples of these patients were tested for possible VL.
infection using several methods including serological tests, microscopy, PCR.

Results: Nineteen (32.2%) patients had positive results for VL after one or more of the tests performed, while only 7 patients (11.8%) had positive results with all the tests including Giemsa stain. Four (6.8%) patients had negative results based on all the serological tests performed except for positive results with Giemsa stain, culture and PCR. The other 4 (6.8%) patients had positive results with Formol-gel, ELISA IgG (>1.1 ISR) and IFAT IgG, (>1/256) but negative results were obtained with direct microscopic examination, culture and PCR. Using PCR Leishmania infantum DNA was detected in 11(18.6%) of the (Leishmania) cultures originated from the bone marrow samples. Plasmodium vivax was found in 2 (3.4%) patients and leptospira was detected in 1 (1.7%) patient. One (1.7%) patient was diagnosed with Pneumonia (Streptococcus pneumoniae). Forty (67.8%) patients had negative results after direct microscopic examination, culture, serological tests and PCR. The kappa coefficients K = 0.80 K = 1.00, K = 0.51, K = 0.55 and K = 0.45 were evaluated for PCR and direct microscopic examination, PCR and culture, PCR and ELISA, PCR and IFAT and PCR and Formol-Gel, as perfect agreement, perfect agreement, moderate agreement and moderate agreement fair moderate, respectively. The probability values (p) for comparisons of all the above tests with PCR showed a significant correlation (p < 0.000)

Conclusion: In conclusion, we found that no single method alone was sufficient enough to diagnose VL accurately; however, combined with PCR, all these methods can reveal better and sensitive results ultimately leading to a correct diagnosis. We also suggest that PCR has to be applied with other laboratory diagnostic tests in order to increase the sensitivity in diagnosis and decrease the possible defects in diagnosis.

doi:10.1016/j.ijid.2010.02.596

82.008

Immunological profile of CD18-deficient mice during Schistosoma mansoni infection

M.S. Espindola*, F.G. Frantz, L.H. Faccioli

Universidade de São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Ribeirão Preto, SP, Brazil

Background: Schistosomiasis is recognized as the most important human helminth infection in terms of morbidity and mortality harboring around 200 million people worldwide, being considered a risk for travelers. A study focusing the role of integrins, which are involved on cellular migration, antigen presentation and T cell activation, is necessary on the knowledge of immunopathology during schistosomiasis. The aim of this work is to evaluate the role of CD18 molecule, a β2 integrin, in modulate the immune response and pathology during the development of experimental schistosomiasis.

Methods: C57BL/6 (WT) mice and CD18low mice were percutaneously infected with 50 cercariae and the parasitological evaluation was done 48 days after infection. The adult worms were recovered from the hepatic portal system and the liver by perfusion with citrate saline. Ten and 48 days after infection, the cellular recruitment to the bronchoalveolar lavage fluid (BALF), as well the number of inflammatory cells present on the peripheral blood and the cytokines production in the lung homogenates were evaluated. To determine the proliferation of T CD3+CD4+ cells and the cytokines production in vitro, splenocytes were stimulated with concanavalin-A.

Results: CD18low mice showed an increased susceptibility to infection with S. mansoni since the worm burden was 135% higher than in the WT group. Nevertheless, the cellular recruitment to the BALF was similar between WT and CD18low mice, while CD18low mice showed a markedly enhancement on the accumulation of mononuclear cells in the peripheral blood, suggesting that less effector cells could migrate through blood to the inflammatory focus. Moreover, T cells from CD18low mice presented reduced potential to proliferate in the presence of Con-A than cells from infected WT mice. Ten days after infection the measurement of TNF-α, IL-12, IL-5, IL-10 and IL-4 in the lung homogenates was always lower in CD18low mice. Although, 48 days after infection, only IL-5 and IL-12 in CD18low mice showed slightly inferior levels. After in vitro stimulation of splenocytes with Con-A, just IL-5 production from CD18low mice was lower than WT.

CD18 low mice are more susceptible to infection with S. mansoni than WT mice. Worm burden was obtained by perfusion of the hepatic portal system with citrate saline. ** p < 0.01

Conclusion: The deficiency of CD18 molecule causes an uncontrolled parasite burden and changes of immune patterns, magnifying the severity of disease.

doi:10.1016/j.ijid.2010.02.597

82.009

Control of Chagas disease patients with chronic form of its treatment after Benznidazole treatment


1 Hospital General Universitario de Valencia, Valencia, Spain
2 Centro de transfusión de la CV, Valencia, Spain
3 CHGUV, Valencia, Spain

Background: Chagas disease is caused by the parasite Trypanosoma cruzi (TC). It’s estimated that around sixteen million people are infected in Latin America and represents a serious blood safety problem due to increasing immigration from these countries. Following the acute phase of the