Journal of Microbiology, Immunology and Infection (2017) xx, 1–11

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Cisplatin along with herbal drug treatment reduces the percentage of regulatory T cells and decreased the severity of experimental visceral leishmaniasis

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Received 24 July 2016; received in revised form 4 January 2017; accepted 13 March 2017
Available online ■ ■ ■

KEYWORDS
Asparagus racemosus;
Cisplatin;
Tinospora cordifolia;
T regulatory cells;
Withania somnifera

Abstract *Background:* Visceral leishmaniasis is the most alarming and devastating amongst the various forms of leishmaniases. It is caused by *Leishmania donovani*, an obligate intracellular parasite of macrophages that survives through immunosuppression. Absence of T regulatory cells provides complete clearance of the parasite. A few immunoprophylactics have been sought to battle instinctive leishmaniasis, with fluctuating achievement. Our previous studies have shown that treatment of *L. donovani* infected mice with cisplatin along with herbal drugs resulted in decreased parasite load with heightened delayed type hypersensitivity responses (DTH), increased levels of IgG2a, IFN-γ, IL-2, CD4+ cells, NK 1.1 cells over that of IgG1, IL-4, 1L-10, CD8+ and CD19 in infected mice.

Methods: Along the above lines, the present study further evaluated the percentage of CD4+ CD25+ FoxP3+ T regulatory cells and ultra structural changes in kidney, liver and spleen. Cisplatin (5 mg/kg b.wt. daily for 5 days, i.p.) along with *Tinospora cordifolia* (100 mg/kg b.wt. daily for 15 days, p.o.) or *Withania somnifera* (350 mg/kg b.wt. daily for 15 days, p.o.) or *Asparagus racemosus* (650 mg/kg b.wt. daily for 15 days, p.o.) was administered to *L. donovani* infected BALB/c and after 30 days post treatment mice were sacrificed.

Results: The findings uncover a significant reduction in parasite load coupled with decreased percentage of Treg cells and no pathological changes at ultra structural level.

Conclusion: In this manner, results acquired recommend that the decrease in percentage of T reg cells may further help the antileishmanial remedial impact of cisplatin alongside natural medications.

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http://dx.doi.org/10.1016/j.jmii.2017.03.001

Please cite this article in press as: Sachdeva H, Kaur S, Cisplatin along with herbal drug treatment reduces the percentage of regulatory T cells and decreased the severity of experimental visceral leishmaniasis, Journal of Microbiology, Immunology and Infection (2017), http://dx.doi.org/10.1016/j.jmii.2017.03.001

Introduction

Visceral leishmaniasis (VL) is a life threatening systemic disease caused by the parasite *L. donovani* and is positioned by World Health Organization as the second most paramount disease after malaria.[1] It is a significant reason of morbidity and mortality in East Africa and the Indian subcontinent.[2] 90 percent of worldwide VL cases occur in Bangladesh, India (mainly northeastern region) Nepal, Sudan, Ethiopia and Brazil.[3] In Indian subcontinent, the disease has been reported from 109 districts (45 in Bangladesh, 52 in India and 12 in Nepal) with yearly occurrence of 136,500, 270,000 and 12, 600 cases in Bangladesh, India and Nepal respectively.[4]

Treatment options for VL are limited. Pentavalent antimonials have viably served as the therapeutic mainstay against leishmaniasis, yet reports of large-scale resistance in India have required assessment of alternative therapeutic modalities.[5] These include amphotericin B together with its liposomal formulation, paromomycin and sitamaquine, however, each have their own limitations of affordability and toxicity and require parenteral administration. Even the orally effective antileishmanial, miltefosine, is associated with gastrointestinal disturbances and teratogenicity.[6]

The disease is portrayed by depressed cell-mediated immunity (CMI) and agents which directly stimulate the macrophage to kill intracellular amastigotes[7] through enhanced release of nitric oxide (NO)[8] and/or induce the basic T-helper type 1 (Th1)-cell anti-leishmania immune response would provide a rationale for treatment in visceral infection.[9] Several studies have already been reported emphasizing the benefits of combination of antileishmanial drugs with immunostimulants.[10]

Cisplatin is a highly efficient anti-neoplastic drug commonly used as a first-line therapy for treatment of various solid tumors.[11] The anticancer effect of cisplatin is mediated by apoptosis and DNA-crosslinks with subsequent cytotoxic lesions in malignant cells.[12] However, its clinical use is associated with dose and duration-dependent nephrotoxic side effect.[13] Generation of reactive oxygen species (ROS), impaired glutathione metabolism, alterations in the mitochondrial antioxidant enzymes and increase in lipid peroxidation are the most plausible mechanisms of cisplatin induced toxicity.[14] Medicinal plants and natural herbal products have potential antioxidant activity and are therefore often administered along with chemotherapeutic agents to provide better protection against their toxic side effects.[15,16]

Cisplatin has been found to have antileishmanial activity *in vitro* at a concentration of 0.25—64 μM.[17] It's *in vivo* antileishmanial activity has also been reported from our laboratory. The treatment of *L. donovani* infected BALB/c mice with cisplatin resulted in decreased parasite load and it was 81.76% on 21 post treatment day (p.t.d.). However, treatment with cispaltin even at low doses generated nephrotoxicity and hepatotoxicity.[18]

Thus, keeping in mind, the immunosuppression caused during visceral leishmaniasis infection and the side effects of the drug cisplatin, we have previously used immunomodulatory and protective herbal drugs *T. cordifolia*, *W. somnifera* and *A. racemosus* along with cisplatin against visceral leishmaniasis. Our results showed decrease in parasite load with enhanced generation of DTH responses, increased levels of Th1 cytokines (IL-2 and IFN-γ), IgG2a, CD4+ T cells and NK 1.1 cells.[19,20a,b]

Evidence from experimental murine models of infection suggests that natural Treg cells promote survival of *Leishmania* parasites and reactivation of disease.[21] Because immunopathology is thought to play a major role in the pathogenesis of active VL and Treg cells are considered important in the regulatory mechanisms of immunity in VL and there are not any reports regarding the percentage of Treg cells prior and after the treatment, thus we herein, extend our studies to explore the percentages of Treg cells amid infection and after the drug treatment. Additionally, pathological changes have furthermore been observed at the ultra structural level.

Methods

Parasite

L. donovani (MHOM/IN/80/Dd8) promastigotes were obtained from the London School of Tropical Hygiene and Medicine, London. The promastigote culture was maintained *in vitro* at 22 ± 1 °C in modified Novy, McNeal and Nicolle's (NNN) medium by serial subcultures after every 48—72 h.

Animals

Inbred BALB/c mice were purchased from the Institute of Microbial Technology, Chandigarh, India and those at 6—8 week of age were used for the experiment. All mice were maintained at controlled temperature and humidity, with a 12 h light-dark cycle, and sterile food and water *ad libitum*. Experiments were carried out according to the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). The ethical clearance for conducting various experiments mentioned in the study on BALB/c mice was taken from Institutional Animal Ethics Committee (IAEC) of the Panjab University, Chandigarh (Approval No. 10/33/CAH). This work was approved by institutional animal ethics Committee.

In vivo infection of mice

The parasites in the logarithmic phase of growth were used for antigen preparation. The culture was pooled and centrifuged for 15 min at 2500 rpm. The supernatant was discarded and the pellet (promastigotes) was washed thrice in PBS. Finally, after last wash, supernatant was discarded and to the remaining pellet, 1 ml of PBS was added. The promastigotes were then counted in the Neubauer's chamber. For this promastigote suspension was diluted in 10% buffered formalin. Promastigotes were then adjusted to a concentration of 10^8 parasites(promastigotes)/ml. Mice were then injected 0.1 ml of this suspension containing 10^7 parasites, intracardially.

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