Experimental Parasitology 137 (2014) 53–65

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Tinospora cordifolia as a protective and immunomodulatory agent in combination with cisplatin against murine visceral leishmaniasis

Heena Sachdeva [a], Rakesh Sehgal [b], Sukhbir Kaur [a,*]

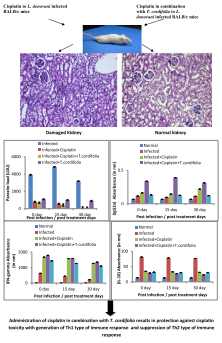
[a] Department of Zoology, Panjab University, Chandigarh 160014, India
[b] Department of Parasitology, Post Graduate Institute of Medical Education and Research, Chandigarh 160014, India

HIGHLIGHTS

- Administration of cisplatin in *L. donovani* infected BALB/c mice resulted in decreased parasite load.
- Cisplatin administration was associated with hepatotoxicity and nephrotoxicity.
- Oral treatment of *T. cordifolia* along with cisplatin ameliorated the cisplatin induced toxicity.
- Combination treatment resulted in the generation of protective Th1 type of immune responses.

GRAPHICAL ABSTRACT

Administration of cisplatin in combination with *T. cordifolia* results in protection against cisplatin toxicity with generation of Th1 type of immune response and suppression of Th2 type of immune response.



ARTICLE INFO

Article history:
Received 3 August 2012
Received in revised form 11 December 2013
Accepted 16 December 2013
Available online 25 December 2013

Keywords:
Visceral leishmaniasis
Cisplatin
Tinospora cordifolia
Hepatoprotective
Nephroprotective
Immunomodulatory
Antileishmanial

ABSTRACT

Effect of pure herb, *Tinospora cordifolia* was studied for its hepatoprotective, nephroprotective and immunomodulatory activity against high dose cisplatin treatment in *Leishmania donovani* infected BALB/c mice. Administration of cisplatin (5 mg/kg b.wt. daily for 5 days, i.p.) reduced the parasite load in *L. donovani* infected BALB/c mice but produced damage in liver and kidney as manifested biochemically by an increase in serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum urea, serum creatinine and various electrolytes etc. These biochemical analyses were further supported by cisplatin induced morphological changes in kidney, liver and spleen. To combat this pure herb, *T. cordifolia* (100 mg/kg b.wt. for 15 days daily) was used in combination with cisplatin in *L. donovani* infected BALB/c mice and it was found that all the aforementioned changes were effectively attenuated by *T. cordifolia* when administered in combination with cisplatin. Moreover, flow cytometric analysis of lymphocyte surface markers of T cells (CD3+, CD4+ and CD8+), NK1.1 and B cells (CD19) indicated prominent enhancement in proliferation and differentiation of lymphocytes. *T. cordifolia* in combination with cisplatin selectively induced Th1 type of immune response as depicted by enhanced levels of IFN-γ and IL-2 whereas Th2 specific cytokines IL-4 and IL-10 observed a moderate decline. Confirmation of Th1 polarization was further obtained from augmented levels of IgG2a over IgG1 and heightened DTH (delayed type hypersensitivity) response. Thus, our results suggest that treatment by *T. cordifolia* may be a critical remedy for the amelioration of adverse effects of cisplatin. Thus, this might serve as a novel combination against visceral leishmaniasis in future.

* Corresponding author. Address: Parasitology Laboratory, Department of Zoology, Panjab University, Chandigarh 160014, India. Fax: +91 172 2541409.
E-mail address: puzoology@yahoo.com (S. Kaur).

0014-4894/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.exppara.2013.12.006

1. Introduction

Leishmania is the causative agent of various forms of leishmaniasis, a significant cause of morbidity and mortality. The clinical manifestations of disease range from self-healing cutaneous and mucocutaneous skin ulcers to fatal visceral form named visceral leishmaniasis (VL) or kala-azar (Ait-Oudhia et al., 2011).

Visceral leishmaniasis caused by the parasite *Leishmania donovani*, is a potentially fatal disease, threatening almost 350 million people in 98 countries (WHO, 2010; Nagill and Kaur, 2011). Most of the approximately 500,000 cases of visceral leishmaniasis reported worldwide affect the rural poor in India, Nepal, Bangladesh, Sudan, and Brazil (Desjeux, 2004). It is characterized by prolonged fever, enlarged spleen and liver, substantial weight loss and progressive anemia.

Current control measures rely on chemotherapy to alleviate disease. However, there is consensus that in the longer term, vaccines ought to become a major tool in the control of this group of diseases. Unfortunately, the development of vaccines has been hampered by significant antigenic diversity (Handman, 2001). One of the available anti-leishmanial drugs, sodium antimony gluconate (SAG), is clinically unsatisfactory because many VL cases are not responsive to it; furthermore, the cases that do respond tend to relapse at a later stage (Thakur et al., 1998; Sundar et al., 2000). Other drugs recommended for the treatment of visceral leishmaniasis have some limitations including development of resistance, parenteral administration, toxic side effects, high costs and long courses of treatment (Perez-Victoria et al., 2003; Sundar and Chatterjee, 2006) viz; 28 days of oral treatment with miltefosine, 30 days infusion with Amphotericin B and 21 days intramascular injections with paromomycin sulfate. The efficacy of drugs is also compromised due to suppression of immune function during the course of infection (Shakya et al., 2011).

Cisplatin [cis-diaminedichloro-platinum (II)], an antitumor DNA binding drug has been found to have antileishmanial activity *in vitro* at a concentration of 0.25–64 μM (Tavaers et al., 2007). *In vivo* antileishmanial activity of cisplatin has also been reported from our laboratory. Treatment with low doses of cisplatin (0.5 mg and 1 mg/kg b.wt.) in *L. donovani* infected BALB/c mice resulted in decreased parasite load but the parasites were not eliminated completely. However, mild toxicity was reported with 1 mg/kg b.wt. of cisplatin as an increase in levels of SGOT, SGPT, BUN, blood urea and creatinine was observed. Moreover, cisplatin treatment did not cause immunosuppression as depicted by increased DTH response to leishmanin which is an indicator of cell-mediated immune responses (Kaur et al., 2010). Increased DTH response is an important factor as for the drug to be effective, the co-operation of the immune system of the host is necessary (Merritt et al., 2003).

Cisplatin has severe toxic effects such as nephrotoxicity and hepatotoxicity that interfere with its therapeutic efficacy. Although the nephrotoxicity of CDDP has been recognized as the most important dose-limiting factor, little is known about CDDP induced liver injury. Oxidative stress injury is actively involved in pathogenesis of cisplatin induced acute kidney injury. Reactive oxygen species directly act on cell components, including lipids, proteins and DNA (Kawai et al., 2006). Hepatotoxicity is not considered as a dose limiting toxicity for CDDP, but liver toxicity can occur when the antineoplastic drug is administered at high doses (Zicca et al., 2004). The investigations revealed a significant increase in lipid peroxidation status and decrease in glutathione level in hepatic tissue of rat after cisplatin treatment (Van Basten et al., 1997). Oxidative stress also appears to play an important role in cisplatin induced hepatotoxicity (Lieber, 1997).

A large No. of studies have focused on measures for preventing cisplatin induced side effects via the simultaneous supplementation of preventive agents (Ali et al., 2006). It is possible to minimize the toxic effect of cisplatin by a compensatory mechanism involving Vitamin E and Vitamin A via induction of antioxidant enzyme activities (Dillioglugil et al., 2005). The ethanol extract of *Curcuma comosa* has been shown to exhibit effective protection against cisplatin-induced nephrotoxicity mediated through its antioxidant activity (Jariyawat et al., 2009). Ethanol extract of *Zingiber officinale* alone and in combination with vitamin E also partially ameliorated cisplatin-induced nephrotoxicity. This protection was mediated either by preventing the cisplatin-induced decline of renal antioxidant defence system or by their direct free radical scavenging activity (Ajith et al., 2007). Lee and his colleagues (2008) studied the protective effect of pericarp extract of *Prunus persica* PPE (peach) against cispatin induced acute toxicity in mice. PPE (500 mg/kg, p.o.) showed a significant protection against the hepatotoxicity and nephrotoxicity induced by single injection of cispaltin (45 mg/kg, i.p.) as PPE significantly inhibited the cisplatin induced elevation in serum alanine aminotransferase and aspartate aminotransferase in liver and serum blood urea nitrogen and creatinie levels in kidneys. In addition administration of PPE caused recovery of nitric oxide and tissue lipid peroxidation. Abdelmeguid and his colleagues (2010) studied the ameliorative effect of silymarin, a plant extract on cisplatin induced hepatotoxicity in rats. They found that pretreatment with silymarin 2 h before cisplatin significantly decreased the pathological changes induced by cisplatin.

T. cordifolia is an important medicinal plant recognized as a vital component of a majority of ayurvedic preparations (Dahanukar et al., 1999; Sinha et al., 2004). Extracts of this plant have been shown to possess many therapeutic properties including general tonic, antiinflammatory, antiarthritic, antimalarial, aphrodisiac (Rao et al., 2008), antiallergic (Nayampalli et al., 1986), antidiabetic (Wadood et al., 1992), antihepatotoxic (Bhupindu et al., 1981; Rege et al., 1984) antipyretic (Kumar and Shrivastav, 1995) and nephroprotective (Khanam et al., 2011). *T. cordifolia* exhibited excellent antioxidant activity in methanol, ethanol and water extract. They were effective in scavenging superoxide anion radical and inhibited deoxyribose degradation induced by hydroxyl radical, scavenging them directly (Bhawya and Anilkumar, 2010).

Various chemical compounds which belong to different classes such as alkaloids, diterpenoids, lactones, glycosides, steroids, sesquiterpenoids, phenolics and aliphatic compounds having immunomodulatory activities have been isolated from this plant (Upadhyay et al., 2010). Also, many low molecular wt. immunomodulators such as clerodane furane diterpene glycoside (TC-1), cordioside (TC-2), syringing (TC-4), cordiofolioside A (TC-5), cordiofolioside B (TC-6) and cordial (TC-7) have been found (Kapil and Sharma, 1997; Singh et al., 2003). A novel polysaccharide termed RR1 (α-D-glucan) exhibiting immune modulating potency has been isolated and its physiological effects and mechanism of macrophage activation in rats has been studied (Nair et al., 2006; Koppada et al., 2009). Berberine, palmatine, tembertarine, magniflorine, choline and tinosporin are reported from stem of *T. cordifolia* (Devprakash et al., 2011). *T. cordifolia* has reported to benefit the immune system in a variety of ways (Rege et al., 1989; Nagarkatti et al., 1994; Kapil and Sharma, 1997).The alcoholic and aqueous extracts of *T. cordifolia* have been tested successfully for immunomodulatory properties (Thatte and Dahanukar, 1989; Rege et al., 1989; Dikshit et al., 2000; Manjrekar et al., 2000).

Since, cisplatin an antineoplastic drug has been reported to have antileishmanial properties but is associated with hepatic and renal disorders, the present study was undertaken to