ABSTRACT

Visceral leishmaniasis (VL) is a neglected tropical disease caused by the protozoan parasite *Leishmania donovani* in India, Bangladesh, China, Nepal and Sudan; by *Leishmania infantum* in North Africa and Southern Europe, and by *Leishmania chagasi* in Latin America. It is mostly endemic in countries that are among the least developed in the world. India, Nepal, and Bangladesh harbor an estimated 67% of the global VL disease burden. The incubation period typically ranges from weeks to months. Stereotypical manifestations of VL include prolonged fever, anemia, splenomegaly, prominent wasting and when untreated death from organ failure and opportunistic infections.

The control of leishmaniasis remains a source of grave concern worldwide. Over the years, control measures have revolved heavily around chemotherapy. However, the treatment of VL is hampered by the intracellular location of the parasite, toxicity of drugs and emergence of drug-resistant strains. The development of effective vaccines represents one of the most promising approaches for providing cost-effective interventions against this disease. However, till date there is no prophylactic vaccine available against any form of human leishmaniasis. Protection in leishmaniasis is dependent on the activation of a Th1 type of immune response with production of a high level of IFN-γ and low levels of IL-4.

It is well established that visceral leishmaniasis causes immunosuppression, so the potential use of drugs that target the host rather than the parasite represents an alternative strategy for combined therapy (immunochemotherapy). The recognition that many anti-leishmanial drugs operate in synergy with host immune mechanisms led us to investigate the antileishmanial efficacy of immunochemotherapy and compare it with chemotherapy and immunotherapy against experimentally induced visceral leishmaniasis infection in BALB/c mice. Animals were infected with $10^7$ promastigotes of *L. donovani* and a month after infection, these animals were given specific immunotherapy (KLD or KLD+MPL-A or 78kDa or 78kDa+MPL-A) or chemotherapy (SSG or cisplatin) or immunochemotherapy (SSG+KLD or SSG+KLD+MPL-A or cisplatin+KLD or cisplatin+KLD+MPL-A or SSG+78kDa or SSG+78kDa+MPL-A or cisplatin+78kDa or cisplatin+78kDa+MPL-A). Animals
were also tested with two different treatments that is one group of animals was treated with a single dose of all the different therapies and another group of animals was treated with two doses of all the above therapies with a gap of 14 days between two doses. The efficacy was assessed by determining the parasite load in impression smears of liver in terms of LDU on different post treatment days. It was found that animals treated with two doses of immunochemotherapy significantly reduced the parasite load in comparison to the chemotherapy or immunotherapy treated animals. Immunochemotherapy with SSG+78kDa+MPL-A was found to be most effective in reducing parasite load against experimental VL, however cisplatin+78kDa+MPL-A was also found comparable to SSG treated animals and therefore can be an alternative option for treatment of VL.

Immunological studies revealed that immunochemotherapy treated animals generated a strong Th1 type of protective immune response as observed by elevated levels of delayed type hypersensitivity (DTH) responses, higher IgG2a levels, lower IgG1 levels and greater cytokine (IFN-γ and IL-2) concentrations compared with chemotherapy or immunotherapy alone.

An initial transient and reversible increase in levels of SGOT, SGPT, BUN, blood urea was observed in infected animals treated with two doses of both the drugs. Though some histopathological changes were also observed in the kidneys of animals treated with two doses, no such change was observed in mice treated with the single dose.

The current study showed that the treatment of infected mice with immunochemotherapy was more effective than chemotherapy or immunotherapy alone in providing protection as well as generation of protective immune responses against experimental visceral leishmaniasis. Thus, it may provide a valid alternative treatment for those cases where conventional chemotherapy is not effective.