ABSTRACT

In Leishmaniasis, the parasites undergo survival and multiplication within the host macrophages - the very cells which normally should have destroyed them. This adaptation on the part of parasites not only deranges host's defense system but even defeats man's ingenuity in directing and delivering drugs to kill them. Moreover, most of the potentially active leishmanicidal compounds are toxic, as also there are some indications of parasites developing resistance and insensitivity to some of the compounds.

Hamsters and Balb/c mice are generally found more suitable than other animals for maintenance of L. donovani parasites, in vivo, for routine screening of potential anti-leishmanial compounds. Acquired resistance to pentavalent antimonials has been demonstrated both experimentally and clinically.

Some of the recent approaches in the chemotherapeutic control of leishmaniasis include targeting of drug through liposomes, development of compounds based on metabolic peculiarities of the parasites and use of various drug combinations. More recently the use of adjuvants (immunomodulators) alone or in combination with parasite antigens has been widely studied with significant results. It has been reported that intravenous administration of glucan, before or after L. donovani infection, causes granulomatous
foci and hypertrophy of Kupffer cells in the liver along with significant reduction in the number of parasites. TDM in water oil emulsion or in aqueous suspension has been shown to confer a significant protective effect against different viral, bacterial and parasitic infections. Similarly, it has been effectively used against tumors.

Combined use of an immunomodulator and a specific drug for therapeutic purposes is a comparatively recent approach to control certain infections. Glucan in combination with ampicillin has been shown to exert a significant synergistic effect on the survival rate of rats with induced bacterial peritonitis. The combined use of sodium stibogluconate and Corynebacterium parvum was reported to stimulate R.E. system in the treatment of L. donovani infected mice. The above regime was found more effective than the administration of drug only, or C. parvum alone. This study for the first time provides a documented evidence that the therapeutic modality involving simultaneous administration of drug and an immunoenhancer can be used to advantage in the treatment of experimental L. donovani infection. BHMARB medium was used for anaemic culture of L. donovani promastigotes. It was found to adequately support the growth of the parasite. The rate of multiplication of promastigotes in BHMARB medium was about 77.83 times after 6 days of incubation. Parasites obtained from 6 days old cultures in BHMARB medium were
washed 6 times in normal saline to which Gentamycin was added. The washed promastigotes were dispensed by shaking and osmolysed in double distilled water. The osmolysed suspension represented the whole antigen preparation from *L. donovani* promastigotes. The soluble and particulate antigen fractions were stained by centrifugation of the whole antigen at 105,000 x g for one hour. The supernatant thus obtained represented the soluble antigen fraction, while the deposit represented the particulate antigen fraction.

The various antigen fractions were partially characterized by estimating protein, carbohydrate, DNA contents and antigenicity before being used for immunological studies. The antigenicity of whole antigen, soluble and particulate antigen fractions was checked against antisera obtained from cases of Kala-azar. Indirect haemagglutination (IHA) and agar diffusion tests were employed to detect the antigenic activity of whole antigen, soluble and particulate antigen fractions. An antigen dilution of 1:16 was found to be the optimum working dilution for use in IHA tests.

Golden hamsters which are highly susceptible to *L. donovani* infection were used for this study. The animals were infected with 1x10^7 promastigotes in 0.1 ml/samples. Two weeks after the inoculation the hamsters were split into different groups. Each group contained 5 hamsters. *L.*
*donovani* infection appeared first in the liver followed by lymphoid organs such as spleen, bonemarrow, lymphnodes and thymus in golden hamsters. Gross pathological changes included hepatomegaly during *L. donovani* infection.

Consequent to the administration of drugs, the response in the infected group was found to be varied depending upon the dosages alone. Drugs given in combination with a TDM dose of 10 mg / kg body weight caused the parasitic count to drop. There was a remarkable drop, when it was combined with 500 ug of TDM per kg body weight.

The haematological studies showed that the total leukocyte count was increased in the infected animals (7408.8) as well as in animals with smaller drug doses (6513.8). When the animals were treated with a combination of lower drug doses and TDM, the count was 5076.6. The mean haemoglobin concentration was found to be decreased in infected animals as well as in the animal groups which were treated with lower drug doses. But when a combined therapy of smaller drug doses and TDM were given, the anaemic condition was overcome and the haemoglobin concentration was increased. The differential leukocyte count was also found to be affected in the infected animals. The lymphocytes, monocytes and the eosionophils were increased in the infected and in smaller drug dose treated animal groups. The count obtained
by using a combination of smaller drug doses and TDM were comparable to those obtained by using higher doses of drugs.

Biochemical assays were carried out in the infected and treated experimental hamsters. Liver showed a significant increase in the level of total lipid, lipid peroxidation, and the total cholesterol content in the infected and in the animal groups which were treated with lesser concentration of drug. The combined therapy of lesser drug doses and TDM normalized the elevated values of the above contents. The level of total proteins, phospholipids, total carbohydrates, total glycogen, glucose and total DNA and RNA were found decreased in the infected and lesser drug dose-treated animal groups. This effect was overcome significantly by the combined therapy of drug and TDM. The altered level of serum transaminase and phosphatase contents indicated the liver dysfunction. The values were normalized by using a combination of drug and TDM and the values obtained by this combined therapy were comparable to the values obtained with higher drug doses alone.

Detection of humoral immune response was carried out by indirect-haemagglutination tests and enzyme-linked immunosorbent assays (ELISA). The highest reciprocal antibody titre was found to be maximum in the infected animal groups, these titre values were found normal after giving the combined therapy of drug and TDM.
The cell-mediated immune response in the experimental animal was demonstrated by the development of delayed type hypersensitivity response (DTH) and macrophage migration inhibition tests. The DTH manifested as footpad swelling following the administration of phenol suspended promastigotes. The hypersensitivity was maximum in the infected and smaller drug dose-treated experimental groups. After the combined therapy and the administration of higher drug doses, the DTH was found to be negative. The maximum macrophage inhibition was found in the infected and in the lesser drug doses treated animal groups. The MMIT appeared to be negative in the higher drug dose treated experimental groups and in the animals which were treated with a combined therapy of drug and TDM.

Further, histopathological studies revealed that the infected liver parenchyma shows patchy necrosis in centrilobular region, indicating hepatocellular damage with an alteration in the pattern of hepatic lobules. Sinusoids were dilated, filled with hypertrophied, kupffer cells containing phagocytosed parasites (L.D. bodies). The animals treated with combined therapy of drug and TDM did not show any parasitic infiltration in the infected liver. Hematoxylin and eosin stained tissue sections from animals given a combined therapy and those receiving higher drug doses, showed normal liver architecture. There was a very
slight inflammation seen but the cells did not contain L.D. bodies.

These findings obtained from various experiments suggest that combined therapy can be gainfully employed in the treatment of *L. donovani* infection. The innate toxicity and the resistance to certain leishmanicidal drugs is an important consideration to use such an immunomodulator, particularly for reducing (substantially) the antileishmanial drug concentration in the treatment of leishmaniasis.