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NANOMEDICINE AND LEISHMANIASIS: FUTURE PROSPECTS

JALAJ KUMAR GOUR[a], ANKITA SRIVASTAVA[a], VINOD KUMAR[a], SURABHI BAJPAI[a], HEMANT KUMAR[b], MANISH MISHRA[b], RAKESH K. SINGH[a*]

[a] *Department of Biochemistry, faculty of Science, Banaras Hindu University, Varanasi-221 005, INDIA*

[b] *Department of Medicine, Institute of Medical sciences, Banaras Hindu University, Varanasi-221 005, INDIA*

The increasing incidences of leishmaniasis due to toxicity and resistance of drug as a result of high drug dose and its spreading to newer areas needt to develop newer drugs for its effective control. Major problem in the treatment of leishmaniasis is failure of humoral response. Moreover, immunodepression and particularly HIV-VL co-infection also present a major cause of treatment failure. Chemotherapy using present antileishmanial drugs is difficult due to the location of parasites within lysosomal vacuoles of the macrophages, which restricts the bioavailability of many potential antileishmanial compounds. So the treatment strategies mainly rely on induction of cellular immune response that can be effectively achieved by targeted drug delivery and the vaccine. Lacuna in its treatment regimen provokes scientists to generate a therapy that is cheaper, requires low dose of drugs and specific to its target of action as well. The causative agent of the disease, an intracellular parasite harbors macrophage and specific tissue sites, which can be the target of drug. Thus targeted drug delivery system is the need of the day. Nowadays the advancement in technology rekindles the hope for the treatment of this disease. This new hope is nanotechnology that employs the use of nanoparticles as drug carriers for the targeted drug delivery. Nanoparticles have proved to be highly stable as well as good carrier capacity as well as reduced toxicity. There are several carriers or drug delivery systems available and for leishmaniasis successful therapy with liposomes have shown some good results. For the researchers to develop the nanomedicine (anti leishmanial drugs) for leishmaniasis is on the top priority in order to get the lesser cost and pain of the patients. This article is an attempt to demonstrate the use of nanomedicine for the benefit of patients suffering with deadly disease leishmaniasis all over the world.

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1. Leishmaniasis

Leishmaniasis is not a single disease; it is a group of diseases with a wide range of clinical manifestations ranging form self-healing cutaneous ulcers to most severe form visceral disease and sometimes even death [1]. It is an infectious disease caused by the protozoan of the genus *Leishmania* which is an obligate intracellular parasite of mammalian macrophages [2]. All forms of leishmaniasis are transmitted to the mammalian macrophages by the bite of certain species of female sand fly during blood meal and responsible for cutaneous leishmnaniasis (CL), mucocutaneous leishmaniais (MCL) or visceral leishmaniasis (VL). Two genera of the sand fly which causes the transmission of disease to human beings are *Lutzomiya* in the new world and *Phlebotomus* in the old world [2]. All the members of the genus *Leishmania* is digenetic and complete its life cycle in two different hosts. The sand fly acts as a primary invertebrate host vector and mammals act as a secondary vertebrate host. The protozoan parasite Leishmania within

the vector sand fly exists as a flagellated, motile promastigote form in the alimentary canal (midgut) and in a non-motile amastigote form within the phagolysosomes of the mammalian macrophages [3, 4]. The flagellated infective stage of the parasite that is metacyclic promastigote form is transmitted to the mammalian host during bite where it transforms itself into the non-motile amastigote forms within the macrophages.

Leishmaniasis is mainly occurring in tropical and sub-tropical countries and about 88 countries are affected by this disease all over the world. Approximately 350 million people live in these areas. Worldwide about 12 million people are affected with 1.5 to 2 million new cases every year [5, 6]. About 1.5 million new cases of cutaneous and 500,000 new cases of visceral leishmaniasis are estimated each year [7]. About 90% cases of visceral leishmaniasis are reported in Bangladesh, Brazil, Nepal, India and Sudan and about same of 90% cases of cutaneous leishmaniasis is found in Brazil, Afghanistan, Iran, Peru, Saudi Arabia, and Syria [8, 9]. In India, the most affected states by visceral leishmaniasis are Bihar, Assam, West Bengal, and eastern Uttar Pradesh, where resistance and relapse against anti-leishmanial drugs are on increase. Recent survey in Bihar (most affected place in India, more than 90% alone) has recorded an alarming 1,000,000 cases with 10,000 unresponsive to antimonials, pentamidine and amphotericin B [10-13].

Visceral leishmaniasis, also known as Indian Kala-azar was first described by Leishman and Donovan in 1903. It is caused by parasite which belongs to the *Leishmania donovani* complex, *L.d donovani, L.d infantum* and *L.d archibaldi* in the old world and *L. d chagasi* in the new world. Various symptoms of Kala-azar are high fever, weight loss, splenomegaly, hepatomegaly etc. [14]. Among various forms of leishmaniasis, visceral leishmaniasis is the most severe form and lethal, if it is left untreated, particularly in patients suffering from AIDS [15]. Another form is, cutaneous form of leishmaniasis often associated with a self healing skin ulcer. It is the most common form which causes a sore at the bite site and caused by *Leishmania major, L. tropica, L. aethiopica* and subspecies of *L. mexicana.* Generally, treatment is not always recommended but if the lesions are present in a cosmetically sensitive area, treatment is recommended. The drug which is generally used for the treatment of cutaneous leishmaniasis for the past 50 years are pentavalent antimony compounds like meglumine antimonite and stibogluconate, but it is proved to be poor clinical agent because of their high levels of toxicity [16]. The pentavalent antimony compounds are also recommended for the treatment of mucocutaneous leishmaniasis at the same dose [17]. However, due to increasing resistance against pentamonial as well as spreading of the disease in the newer areas, total cure of leishmaniasis has not been achieved yet in spite of a lot of treatment choices available. One of the reasons is the high mortality rate of this disease which is very high because of poor nutrition, infection and other stresses; it is very much common among children and poor peoples. Moreover males are more commonly infected than females [18].

The pentamonials are no longer being need in visceral leishmaniasis hyper endemic regions of India because of its declining efficacy. During past few years various drugs like amphotericin B, paromomycin, pentamidine, miltefosine etc. have been used to cure leishmaniasis however, out of these only amphotericin B is promising.

VL is associated with impaired cellular immune responses and sustained humoral response along with high anti-leishmanial antibody production [19, 20]. Humans after infection with Leishmania parasites, usually develop immunity to reinfection. The immnity against visceral leishmaniasis is mainly dependent on CD4+ cells and impaired CD4+ T-cell results into serious complications during leishmaniasis [21]. The immune response against Leishmania parasite basically hinges on two different types of CD4+ T-cell subsets and these come into play after parasite infection i.e. Th_1 and Th_2, which depends on the type of cytokine elicited by the antigen stimulation [22]. The development of either Th_1 or Th_2 cells subset against particular pathogens is associated with immunity [23]. Th_1 cell subset responsible for high production of interferon (IFN-gamma) and interleukin 2 (IL-2), leads to cell mediated immunity along with activation of macrophages whereas, production of IL-4, IL-5, IL-10 are associated with increased humoral immunity and the Th_2 cell subset takes part in immune response [24]. It has been seen that elevated level of IL-12 production during infection responsible for the CD4+ Th_1 response with high IFN-gamma levels secreted by natural killer cells and no IL-4, shows recovery form

infection, on the other hand, production of IL-4 instead of IL-12 during infection causes Th_2 response and results into disease progression [25].

2. Nanoparticles in the treatment of leishmaniasis

Nanomedicine is defined as the medical application of nanotechnology to human health. The field of nanomedicine has a wide approach ranging from medical use of nanomaterials to nanoelectronic biosensors and even molecular nanotechnology [26]. Uses of nanoparticles in different diseases were discussed widely now a day [26-34]. Nanoparticles like emulsomes, liposomes and nanospheres have been of great importance for drug delivery as drug carriers [35]. Among several nanoparticles implementing for treatment, liposomes are the best studied for evaluating the role of leishmanial antigens as well as efficacy of antileishmanial activity of compounds as compared to any other parasitic disease mainly due to the fact that Leishmania parasite resides within the macrophages which are responsible for clearance of liposomes in vivo. Liposomal formulation with drug or antigen has been proved to be successful against leishmaniasis. Moreover, the use of conventional liposomes with anti leishmanial drugs has been proved to be associated with the reduction in their toxicity profile [35]. Gupta et al reviewed that till date the most extensively studied carriers for DDS are liposomes and microspheres. The advantage of these carriers lies in the fact that their efficacy and specificity can be altered to a great extent by modulating their physicochemical properties that which includes hydrophilicity, surface charge, composition, concentration, and the key factor of their success is the presence of various target specific ligands on their surface [36]. Macrophages act as host cells for many parasites and bacteria, which give rise to outbreak of so many deadly diseases (eg. leishmaniasis, tuberculosis etc. and its surface represents various receptors for pathogens bearing ligands and are the main target of drug action. Thus macrophage-specific delivery systems are the focus of much interest nowadays. Moreover, the macrophage surface contains receptors that recognize terminal galactose, mannose, fucose or glucose residues of glycosides therefore sugar bearing liposomes were designed for improvement in macrophage targeting of anti-leishmanial agents [36]. Furthermore, mannose-grafted liposomal form was more efficient in transporting the drug to macrophages [37]. In addition, macrophages upon interaction with particulate drug delivery vehicles may act as secondary drug repository and contribute in localized delivery of the drug at the infected site [36]. Nanoparticles are also purposed for vaccine delivery. The vaccine-delivery systems comprise emulsions, microparticles and liposomes. Progressively more sophisticated drug delivery systems are being developed in which immuno-stimulatory adjuvant can be incorporated with the antigen. The benefit of this approach is to make sure that both the antigen and adjuvant are delivered into the same population of antigen-presenting cells. But the larger size, stability and storage are being the common problem of liposome therapy. Therefore non-ionic surfactant vesicles, niosomes were prepared, for their different drug distribution and release characteristics compared to liposomes. When tested in vivo, the retention capacity of niosomes was found to be higher than that of liposomes due to the absence of lipid molecules and their smaller size [38]. Another nanaoparticle employed for drug delivery system (DDS) is immunoliposomes a combination of antibody coupled to liposomes and the activity IgG coupled liposomes was found 2-3 times more than free IgG against several strains of leishmania [35]. New approaches are being made in order to make DDS more advanced. In this respect, DNA vaccines is an emerging optimism. It may serve as a potential vaccine for important pathogens such as HIV, hepatitis C, tuberculosis, and malaria. It is well known that the expression of an antigen or antigens from plasmid DNA (pDNA) may elicit both humoral and cellular immune responses. Therefore, there exists a clear need for new vaccine delivery systems that can be administered at low doses to elicit strong humoral and cellular immune responses. Thus development of microparticles and nanoparticles as delivery systems for DNA vaccines is a hopeful approach [39]. Therefore to construct vaccine against leishmaniasis, efficacy of any leishmanial antigen must be tested in order to elicit proper immune response. To evaluate the antigenicity of LAg with or without adjuvant liposomes are successful delivery system.

3. Future Prospects of Nanotechnology in Leishmaniasis

In spite of several treatment options, the frequency of leishmaniasis is on increase because of its spreading in non endemic as well as newer areas. Further, the available drugs are facing the problem of side effects, toxicity, unavailability high cost as well as resistance of parasite due to high drug dose. Moreover the silent entry of parasite and its residence within macrophage is responsible for its rescue. The targeted drug delivery system provides the facility of administration of low dose of drug and target specific activity could be achieved using nanotechnology which comprises the use of nano scaled particles loaded with drug of interest or antigens/adjutants, if vaccine is to be formulated. This technology may prove to be superior than current treatment modalities including reduced cost of drug, improved bioavailability and lower drug toxicity which enhance the patient adherence to treatment. The use of nanotechnology for treatment of leishmaniasis has showed promising results which could be the pavement for curing this disease. The increasing interest in this field of drug research demonstrates the unspoken assurance and potential of nanotechnology derived drug delivery systems.

4. Conclusions

Currently there is no drug or vaccine available which is potentially effective or gives satisfactorily result against the deadly disease leishmaniasis. All over the world, scientists are focusing to develop such a potent drug or vaccine using nanotechnology which can be an effective tool against the deadly disease leishmaniasis and will take short duration of time to cure and cost effective which gives the benefit to every common people who is suffering from leishmaniasis all over the world. Drug delivery systems (DDSs) allow the adverse effects caused by problematic routes of administration to be avoided as well as enhancing the antileishmanial activity and reducing the toxicity of the medication. This can be achieved by use of nanoparticles in the treatment of leishmaniasis.

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