ISSN: 2591-7846

In vitro effect of Artemether-Loaded Nanostructured lipid Carrier (NLC) on *Leishmania* infantum

Meisam Khazaei1, Vahid Rahnama[1], Mohammad Motazedian[1, 2,] Soliman Mohammadi Samani[3], Gholamreza Hatam1

[1]Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[2]Nanomedicine and Nano biology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[3]Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

***Visceral leishmaniasis* (VL) is an acute and deadly form of *Leishmaniasis*, caused by *Leishmania* infantum parasite. Due to the toxicity and side effects of conventional treatment options, such as Glucantime and other pentavalent drugs, finding novel drugs with fewer adverse effects is required. Artemether (ART) is one of the derivatives of Artemisinin, which was shown to be effective in treating malaria and more recently, *leishmaniasis*.**

In this study, we compared the effect of ART and nanostructure loaded with artemether (NLC-ART) on Leishmania infantum promastigotes and amastigotes, at different concentrations (2.5-5-10-25-50-100 µg/mL) using the MTT assay method after 24 and 48 hours of treatment.

IC50 values (µg/mL) of promastigote and amastigote of L. infantum to ART/ NLC-ART after 48 hours of treatment were found to be 37.12/32.1 and 16.43/15.42, respectively. Moreover, we found that (NLC-ART), had the lowest cytotoxicity against the J774 macrophage cell line.

Conclusion: The NLC-ART can be a good candidate for the treatment of visceral leishmaniasis.

Keywords: *Leishmania infantum; Artemether; MTT assay; Nanostructured lipid carrier (NLC); Drug delivery*

Accepted March 18, 2021

Introduction

Leishmaniasis is an important vector-borne parasite disease that approximately infects 2 million people globally every year. Amastigotes of this parasite inoculate in the mammal's skin, especially humans by phlebotomine sandflies[1]. *Leishmaniasis* is clinically manifested in three forms; cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis(MCL), and visceral *leishmaniasis*(VL). *Leishmania* infantum and *Leishmania* donovani are the 2 species that can cause VL[2]. Visceral l*eishmaniasis* is highly endemic in countries, such as India, Nepal, Sudan, and Brazil [3]. In Iran, approximately 15,000 new cases occur annually, with the highest incident rate among children below 2 years of age [4, 5]. The main hosts for this disease are stray dogs, causing various symptoms, including irregular fever, lymphadenopathy and lymphocytosis and monocytosis (20-25%)[6]. In humans, *leishmaniasis* symptoms are fever, cough, weight loss, and *hepatosplenomegaly*. In patients with immune deficiency, VL can become a life-threatening problem [7]. The death rate in untreated VL is about 75-95% [6]. The gold standard for the diagnosis of *leishmaniasis* is using microscopic and observational methods to detect amastigotes in the splenic and bone marrow aspiration. Serologic antibody and antigen tests are also used for the diagnosing [8]. Moreover, molecular methods such as PCR are used to detect *Leishmania* parasite DNA in the specimens collected from old and weak scars [9].

Leishmania parasite DNA in the specimens collected from old and weak scars [9]. Generally, antimonial pentavalent drugs are used as the first line of treatment for leishmaniasis. However, the major problems associated with the use of these drugs are the development of drug resistance and various side effects such as renal toxicity, hypotension, pancreatitis, anemia, leukopenia, thrombocytopenia, reversible renal insufficiency, and cardiotoxicity. Amphotericin B and pentamidine are in the second line of treatment, but due to their high toxicity and cost are the main problems of these drugs they are not widely used [10,11, 12]. Recently, it was shown that artemisinin, the anti-malarial herbaceous drug, and its derivatives such as artemether can be used for the treatment of Leishmaniosis and other parasitic diseases, such as schistosomiasis. The effects of artemisinin and its derivatives, including artemether, on treating VL and Leishmania infantum have been previously demonstrated [13]. In the present study, a nanostructured lipid carrier loaded with artemether, (NLC-ART), was evaluated in vitro for the treatment of visceral leishmaniasis.

Materials and Methods

Parasite Culture

L. infantum standard strain (Mcan/IR/07/Moheb/-gh) was obtained from Shiraz University of Medical Sciences, Department of Parasitology and Mycology. Promastigotes

of the parasite were cultured in RPMI 1640 enriched with FBS 15% (v/v) (fetal bovine serum), 100 IU/mL of penicillin, and 100 μg/mL of streptomycin, and then incubated in 24-26°C.

J774 cells culture

In this study, we used murine macrophage cell, (J774), obtained from Shiraz University of Medical Sciences Department of Immunology.

We cultured the cells in DMEM enriched with FBS 10% (fetal bovine serum, 100 IU/mL of penicillin, and 100 μg/mL of streptomycin) and then seeded in 24-well plates and incubated at 37°C and 5% CO2. Cells were nurtured white DMEM 10% after 17 h.

Preparation of nanostructure loaded with artemether (NLC-ART)

NLC loaded with artemether (NLC-ART) was obtained from Shiraz University of Medical Sciences, Department of Pharmaceutics School of Pharmacy, which was prepared during an unpublished student research project (16141-01-01-1396.

Statistical analysis

Graph Pad Prism 6 Demo was used for the statistical analysis. P-value less of than 0.05 was considered to be statistically significant. Data were analyzed using the ANOVA test.

Results

Anti-leishmanial activity of the formulations against promastigotes and amastigotes

Using MTT assay, we measured the viability of Leishmania infantum promastigotes in the presence of drugs after 24 h and 48 h of treatment at different concentrations (2.5-5-10-25-50-100 μg mL). As shown in (Figure 1 & 2), NLC-ART had the greatest effect on promastigotes at a concentration of 50 and 100 μg/mL during 24 h and 48 h of treatment. The P values for all concentrations at 24 h and 48 h. MTT test results on drug-treated Leishmania infantum promastigotes after 24 hours indicated a significant difference between NLC-ART, and ART ($P<0.05$). Moreover, the difference remained significant after 48 hours.

Half-maximal inhibitory concentration (IC50) values of promastigotes and amastigotes to all drug formulations are summarized. IC50 values of promastigotes/amastigotes for NLC-ART, after 24 h and 48 h treatment, were obtained as, 31.52 / 16.43 and 27.95 / 15.42 μg/mL, respectively.

Anti-leishmanial activity of the formulations against amastigotes

Next, we assessed the anti-leishmanial activity of the formulations on j774 macrophage cell line infected with "*Leishmania promastigotes*". After 48 hours of culturing the infected j774 cells in the presence of drugs at different concentrations, macrophage viability and IC50 of amastigotes were determined.

While NLC-ART showed negligible toxicity on macrophage cells, it was more effective in targeting the amastigotes residing inside the macrophage cells as compared to ART .IC50 (μg/mL) for the NLC-ART: 27.95 (24 h) and 15.42 (48 h); for the ART: 45.2 (24 h) and 32.1 (48 h).

IC50, CC50, and Selectivity Index (SI) of promastigotes and amastigotes and all formulations are reported.

Discussion

Visceral leishmaniasis is the most serious form of leishmaniasis disease that can be life-threatening if not treated. Currently, antimony pentavalent, Amphotericin B, and pentamidine are among the drugs used for the treatment of VL. However, drug resistance and dangerous side effects are the major unsolved issues associated with these drugs. Therefore, discovering new drugs and/or new methods of drug delivery with low side effects and high therapeutic potential is essential.

The traditional antileishmanial drugs suffer from the long duration of treatment, difficulty of administration and low tolerability. In facing these challenges, nanotechnology can open a new avenue of therapies [14]. With the help of nanotechnology, drugs can be loaded onto well-organized nano carrier systems and be delivered to the site of interest. These nano carrier systems protect the drug from being metabolized, increase the bioavailability, and reduce the toxicity of drugs [15].

Artemether has recently been used for the treatment of Leishmania and other parasitic diseases. With its low toxicity and good efficacy, artemether could be a suitable candidate for the treatment of leishmaniasis. Artemether induces cell death following activation in the presence of iron and production of free radicals [15].

In recent years, several studies have addressed the effect of artemisinin and its derivatives on Leishmaniasis. In an in-vivo study on BALB/c mice infected with Leishmania infantum. Described a significant reduction of parasite burden and splenic weight loss following treatment.

An in-vitro study reported that artemisinin was effective in eliminating the Leishmania major parasite through apoptosis induction of promastigotes [16]. Antileishmanial activity and toxicity of artemether were also studied in an in-vitro study. They showed that the artemether had the ability to inhibit the growth of intracellular and extracellular residing Leishmania major. The same group also studied the effect of artemether ointment on BALB/c mice lesions following infection with Leishmania. The result showed that the artemether significantly decreased the diameter of the lesions [17]. The effect of artemether administration was evaluated on infected mice by the L.infantum parasite. And, accordingly the parasite burden decreased in the liver and spleen following oral treatment [13]. In this study, we investigated the effect of a nanostructured lipid carrier loaded with artemether (NLC-ART) on treating the visceral leishmaniasis. The elimination of amastigotes within macrophages showed that the NLC-ART drug was able to pass through the macrophage membrane barrier; and doing so more effectively that free ART.

We are currently focusing on the in-vivo evaluation of NLC encapsulated drug in treating the visceral form of the Leishmaniasis. Based on our findings presented in this study and our future in-vivo assessment of the drug, we are hoping to introduce NLC-ART as a potent drug to be used in clinical trials for treating this disease.

Conclusion

In our study, we took advantage of nanotechnology in producing a new form of antileishmanial drug Artemether. Nanostructured lipid carriers loaded with artemether were found to be more effective in eliminating the amastigotes and promastigote infected macrophages. Importantly, the NLC and NLC-ATR had much lower toxicity than amphotericin B (as a positive control), and exhibited no toxicity to macrophage cells.

By examining the level of CC50 for macrophages infected with amastigotes, we concluded that our drug passed from the cellular barriers freely, entered inside the infected macrophages, and showed a promising effect eliminating the infection.

Acknowledgements

The results presented in this research were extracted from Meisam khazaei M.Sc. student. The Dissertation was supported by the Research Council of Shiraz University of Medical Sciences.

Authors' contributions

All authors contributed in this study: Parasite and cell Culture, Statistical analysis, Promastigote In vitro cytotoxicity assay, J774 macrophages in vitro cytotoxicity assay, data collection, [Meisam khazaei,vahid rahnama,Mohammad Motazedian, Soliman Mohammadi Samani, Gholamreza Hatam], Preparation of nano structure loaded with artemether (NLC-ART)[vahid rahnama].

References

1. Asgari Q, Gholizadeh F, Nohtani M, et al. Cutaneous leishmaniasis associated with Systemic Lupus Erythematosus (SLE). Le infezioni in medicina 2019; 27(3):345-349.
2. Mohebali M, Malmasi A, Hajjaran H, et al. Disseminated Leishmaniasis Caused by Leishmania tropica in a Puppy from Karaj, Central Iran. Iran J Parasitol. 2011; 6(2): 69–73.
3. Murray E. Challenges in educational research. ASME. 2002; 36(2).
4. Alborzi A, Pouladfar GR, Aalami MH. Visceral leishmaniasis; literature review and Iranian experience. Iranian Journal of Clinical Infectious Diseases. 2007; 2(2):99-108.
5. Alborzi A, Rasouli M, Shamsizadeh A. Leishmania tropica–isolated patient with visceral leishmaniasis in southern Iran. Am J Trop Med Hyg 2006; 74(2):306-307.
6. John D, Petri W. Markell and Voge's Medical Parasitology. Greg Martin. 9th Edition. 2006:408.
7. Desjeux P. The increase in risk factors for leishmaniasis worldwide. Trans. R. Soc. Trop. 2001; 95(3): 239-243.
8. Singh S, Ramu S. Recent advances in the diagnosis of leishmaniasis. J. Postgrad. Med. 49(1):55-60.
9. Costa L. et al. Evaluation of PCR in the diagnosis of canine leishmaniasis in two different epidemiological regions: Campinas (SP) and Teresina (PI), Brazil. Epidemiology & Infection 2015; 143(5):1088-1095.
10. Basselin M, Denise H, Coombs GH, Barrett MP. Resistance to pentamidine in Leishmania mexicana involves exclusion of the drug from the mitochondrion. AAC 2002; 46(12): 3731-3738.
11. Burgess JL, Birchall R. Nephrotoxicity of amphotericin B, with emphasis on changes in tubular function. AJM. 1972; 53(1):77-84.
12. Croft S, Neal R, Pendergast W, Chan J. The activity of alkyl phosphorylcholines and related derivatives against Leishmania donovani. BCP. 1987; 36(16):2633-2636.
13. Dehkordi NM, Ghaffarifar F, Hassan ZM, Heydari FE. In vitro and in vivo studies of anti leishmanial effect of artemether on Leishmania infantum. Jundishapur J Microbiol. 2013;6(5).
14. Benita S. Microencapsulation: methods and industrial applications. Crc Press 2005.
15. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. Pharmaceutical research. 2016; 33(10):2373-2387.
16. Dehkordi NM, Ghaffarifar F, Hassan ZM, Heydari FE. In vitro and in vivo studies of anti leishmanial effect of artemether on Leishmania infantum. Jundishapur J Microbiol 2013; 6(5).
17. Fathi H, Ebrahimzadeh. Antioxidant and free radical scavenging activities of Hypericum perforatum L. (St. John's wort). IJFSE. 2013; 3(2): 68-72.

*Correspondence to:

Meisam Khazaei

Department of Parasitology and Mycology

School of Medicine, Shiraz University of Medical Sciences Shiraz
Iran