Stealth liposomal formulation for the treatment of visceral leishmaniosis: preparation, characterization and in vitro therapeutic effectiveness

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Visceral leishmaniasis (VL) is a disease which represents serious public health problem, if undiagnosed and treated in time, can be lethal to the patient. An estimated 350 million people are at risk of infection with an average of 2 million new cases reported annually including about 50 thousand deaths due to VL. The treatment of infected patients still remains the first line control measure of VL, and the main drugs used to treatment are pentavalent antimonials (MA), amphotericin B, miltefosina (HePC) or pentamidine. Despite the large scale use, especially MA and HePC, these drugs present several limitations, such as collateral effects and reduced efficacy in some cases. Studies have suggested new therapeutic approaches for the treatment of VL based on rejuvenation therapies, such as the use of leishmanicidal drugs in prolonged blood circulation liposomal formulations (stealth liposomes). This strategy could increase the therapeutic efficacy of classical leishmanicidal drugs and diminish its side effects. The aim of this study was to prepare, characterize and determine the in vitro activity of a stealth liposomal formulation containing HePC and MA, for treatment of VL. In order to obtain stealth liposomes containing HePC co-encapsulated with MA (HePC-PEG/AM), DSPE-PEG 2000 polymer was added in the composition of the conventional liposomal vesicle composed by cholesterol, dicetylphosphate and dipalmitoylphosphocholine. The stealth liposome was prepared by the "Dehydration and rehydration of the vesicles" method and the mean of hydrodynamic diameter, zeta potential (Z) and polydispersity index (PI) of vesicles were determined by photon correlation spectroscopy. MA encapsulation efficiency and Sb concentration were determined by electrothermal atomic absorption spectrometry. The mean of diameter of the HePC-PEG/MA vesicles was 200.85nm and formulation was monodispersed (PI= 0.074) and presented excellent electrical stability (z = -66mV). HePC-PEG/MA showed $CC_{50,}$ determined by the colorimetric method MTT, equal 105.06 µg/ml, suggesting lower cellular toxicity of the drug in liposomal formulation. The value of CI_{50} determined by the colorimetric test of resazurin on WHO reference strain *Leishmania infantum* (MHOM/BR/1967/BH46) was 32.65 µg/ml, similar to free HePC (34.23 µg/mL), suggesting that there was no reduction of leishmanicidal activity of HePC in the formulation. The results of the anti intracellular amastigotes assay in infected macrophages (RAW 264.7) showed that the stealth liposomes significantly reduced the macrophages infection rates when compared to the control and treated macrophages with MA. In conclusion, these results are the

proof of concept that an innovative nanostrutured stealth liposomal formulation containing HePC and MA presents characteristics of stability, monodispersion, biocompatibility and induced significant reduction of *L. infantum* infection rates in vitro.

Keywords: Visceral leishmaniasis, treatment, liposome, meglumine antimoniate, miltefosine.

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