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Hyaluronic acid-Amphotericin B Nanocomplexes—A Promising Anti-Leishmanial Targeted Drug Delivery System

Ricardo Carvalho[1], Catarina Gonçalves[2], Ana Isabel Bourbon[2], Lorenzo Pastrana[2], Pier Parpot[3,1], Ana Tomás[4], Miguel Gama[1]

CEB – Centre of Biological Engineering, University of Minho[1], Food Processing group, International Iberian Nanotechnology Laboratory[2], Centre of Chemistry, University of Minho[3], I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto and IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto[4]

Leishmaniasis has been classified as one of the most neglected tropical diseases, causing 50 thousand deaths and 1.5 to 2 million new cases every year, according to the World Health Organization. This disease, promoted by protozoan parasites of the genus *Leishmania*, has a high incidence affecting 89 countries worldwide. Nowadays, current treatment strategies still rely on the antifungal agent amphotericin B (AmB) but are rather inadequate due to the high prevalence of the disease within low-income population of sub-developed regions, the intracellular location of the parasite and the emergence of parasite resistance. Thus, other strategies have been pursued to improve the therapeutic efficacy and to reduce the toxicity of AmB such as the use of biocompatible polysaccharides as carriers. In this work, a simple and inexpensive production process using hyaluronic acid (HA, 50 kDa) was used in order to develop water-soluble hyaluronic acid-amphotericin B nanocomplex (HA-AmB). HA is the main ligand of CD44 receptor, thus being favorably internalized by macrophages that overexpress this receptor upon infection. Therefore, HA arises as a suitable polysaccharide to target the AmB delivery to the leishmania-infected macrophages.

The nanocomplex, obtained by simply processing the mixture of the polysaccharide with the drug in a nanospray dryer (HA-AmB SD), was characterized in terms of size/zeta potential (DLS) and morphology (SEM and Cryo-SEM). Furthermore, an HPLC-MS detection method was optimized and used to determine the AmB content in the nanocomplex. Also, to ascertain the interaction between AmB and the HA, FTIR, DSC and PXRD analysis were performed. Cytotoxic and hemolytic effects were assessed on different cell lines through the resazurin test and in dog's blood, respectively. Anti-leishmanial activity was assessed *in vitro* in axenic cultures of Leishmania by resazurin and in infected bone marrow-derived macrophages (BMMΦ) stained with different fluorescent probes using high-content microscopy.

Our results shown that the produced material has a spherical morphology in aqueous solution with a mean hydrodynamic diameter of 318.4 ± 34.7 nm and low polydispersity (0.239 ± 0.02). Moreover, this material that presents an AmB content of 13.56 ± 3.49 %, has a good colloidal stability due to the highly negative surface charge (-39.45 ± 1.12 mV). DSC and PXRD analysis strongly suggested the formation of an amorphous inclusion complex between AmB and the complex polysaccharide chain networks, explaining the high solubility of the drug in water. The *in vitro* assays showed that compared to free-AmB, the nanocomplex had significantly less cytotoxicity against BMMΦ and HEK293T cell lines, significant less hemolytic effect and inhibited the infection in the Leishmania-infected BMMΦ. Exploratory *in vivo* assays are being conducted in mice. In conclusion, this work has shown that the hyaluronic acid-AmB

nanocomplex is a promising system for the treatment of Leishmaniasis, possessing similar effects to the free-AmB against Leishmania-infected macrophages and Leishmania axenic cultures, with reduced cytotoxicity. Given the affordability, simplicity, low-toxicity and facile scale up of the developed formulation, the hyaluronic acid-AmB nanocomplex may represent an alternative to the expensive nanoformulations available.

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