TITLE: P10 PEPTIDE COMPLEXED IN POLYMERIC NANOPARTICLES AS INTRANASAL THERAPEUTIC VACCINE FOR THE TREATMENT OF PARACOCCIDIOIDOMYCOSIS IN MURINE MODEL.

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ABSTRACT:
Paracoccidioidomycosis (PCM) is a systemic fungal disease, caused by the thermo-dimorphic fungal *Paracoccidioides* spp., that mainly affects the lungs. PCM therapy is usually prescribed, depending of the gravity of infection, in two steps, firstly an initial attack for the rapid control of the infection, and secondly a treatment to prevent the proliferation of possible remaining yeasts, avoiding the recurrence of the disease. The main drugs for the treatment of PCM are polyene chemotherapeutics, sulfanilamide compounds and azoles drugs. As an alternative for treating and / or preventing PCM, the use of vaccines has been explored, promoting the production of IFN-γ, inducing this way an Th1 immune response, ideal for the control of the disease. One of the most studied vaccine candidates is the P10 peptide-based vaccine, consisting of a 15 mer amino acids, provenient from the 43 kDa glycoprotein of *P. brasiliensis.* Studies using P10-based strategies for vaccination have been demonstrating really significant results, nevertheless, the P10 peptide short lifetime impairs its effectiveness, due to premature degradation. An alternative to overcome this problem is by its complexation within nanoparticles in order to protect and improve the immunomodulatory effect of the peptide. For the P10 peptide complexation chitosan was chosen due its physico-chemical and biological characteristics, such as: biocompatibility, mucoadhesiveness and relative low cost. The nanoparticles complexed with P10 peptide at different concentrations were produced by the ionic gelation technique, their poly dispersion index (PDI), size, and Zeta potential (Z potential) were analyzed. The encapsulation efficiency was assessed using the Qubit TM Protein Assay Kit and the toxicity verified by hemolysis and cell viability assays with murine J774.16 macrophages. The nanoparticles obtained presented a size of approximately 220 nm, PDI below 0.5 and Zeta potential of approximately + 20 mV. The encapsulation efficiency was greater than 90% and there was no cytotoxicity effects in the first 48 hours. Treatment with the nanoparticles containing different concentrations of P10 peptide was efficient in reducing lung fungal load during murine PCM and as able to reduce from 4 to 20 the usual standard concentration of the peptide P10 peptide. These results show that the nanoparticle is stable, and presents the physico-chemical characteristics desirable for an intranasal vaccine using chitosan as polymer.

Keywords: Paracoccidioidomycosis, Paracoccidioides spp, Nanoparticles, Chitosan, P10 peptide, Intranasal vaccine.
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