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PLGA nanospheres loaded with autoclaved *Leishmania major* (ALM) and CpG-ODN: Preparation and *in vitro* characterization

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Background and Aims: Several antigens, adjuvants and delivery systems have been evaluated for induction of protective immune responses against Leishmaniasis, but most of them have been inefficient. In this study, PLGA nanospheres as antigen delivery system CpG-ODN as an immunoadjuvant for increasing the immune responses against Autoclaved Leishmania major (ALM) were prepared and characterized.
Methods: PLGA nanospheres prepared by a double-emulsion (W/O/W) technique. The internal aqueous phase contained ALM and CpG-ODN, while the oily phase contained the solution of PLGA in dichloromethan and the external aqueous phase was PVA 7.5% (W/V) solution. Particulate characteristics were studied by scanning electron microscopy and particle size analysis. The encapsulation efficiency was determined by Lowry method for ALM and UV spectroscopy at 260 nm for CpG-ODN. The release profiles of antigen and CpG-ODN from nanospheres evaluated for one week.
Results: Nanospheres were spherical in shape, having smooth surfaces. Mean diameters for blank and ALM + CpG-ODN loaded nanospheres recorded as 302±129 and 300±128 nm respectively. Also, the encapsulation efficiencies of ALM and CpG-ODN were 71.6±8.8 and 49.1±2.4%, respectively. Evaluation of the release profiles of ALM and CpG-ODN from nanospheres showed that 44.8±0.8% of ALM and 29.5±0.2% of CpG-ODN released from nanospheres in one week.
Conclusions: The prepared nanospheres with desirable size, encapsulation efficiency, and slow rate of release, had acceptable features for future in vivo studies.

Keywords: *Leishmania major*; CpG-ODN; PLGA nanosphere; Vaccine