VAC.06 - Upstream and downstream processing, stability and characterization studies of LigANI fragment – process development of a vaccine candidate

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Introduction: Leptospirosis is one of the most common zoonosis in the world according to WHO and the number of leptospirosis cases has been increasing over the years. Our group has been working with Leptospiral immunoglobulin-like proteins as diagnostic and subunit vaccine candidate. Initially, we cloned, expressed and purified different fragments of Lig proteins and in 2007, it was demonstrated the potential of the non-identical LigA region (LigANI) as a vaccine candidate, as it was able to protect 67-100% of the animals against lethal challenge, other groups also demonstrated the potential of this fragment as a vaccine candidate.

Objective: The aim of this work was to establish the upstream and downstream conditions in order to obtain the recombinant LigANI protein with a high degree of purity and homogeneity and to determine the physico-chemical and biological characteristics of the LigANI protein, since these information are essential for the development of a product with biotherapeutic application.

Methodology: We tested the best upstream conditions varying pre- induction time and inductor (IPTG) concentration by central composite design. The optimal results were transferred to a 2 liter-bioreactor maintaining dissolved Oxygen (DO) concentration. For downstream processing, cell lysis was performed using a homogeneizer. The lysate was centrifuged and the supernatant was purified by immobilized metal affinity chromatography (IMAC) evaluating Sodium Chloride concentration. Tangential flow filtration and gel filtration (GF) chromatography were tested for protein desalting. A further polishing step was performed by ion exchange chromatography (IEX) and the purified protein was stored for characterization and stability studies using the following techniques: SDS-PAGE, SEC, Isoelectric focusing, Spectrofluorimetry and Circular dichroism.

Results: Initially, we performed the scale up from 80 mL bioreactor to 2 liters of culture and obtained approximately 1 g of LigANI per liter of culture. The protein was purified using three chromatographic steps: IMAC, GF and IEX and the total yield of the process was around 220 mg of LigANI per liter of culture with a purity of 99.70 ± 0.24%. The experimental data confirmed the molecular mass of 66.9 kDa, isoelectric point of 6.94 and a predominance of β sheet secondary structure. LigANI protein bound to different extracellular matrix proteins and was antigenic against sera from leptospirosis patients. It also demonstrated cross-reactivity with of other pathogenic Leptospiras as Leptospira interrogans serovar Copenhageni anti-LigANI hyperimmune serum recognized LigANI protein from different serovars. Stability data showed that the protein remained stable when stored at -80°C and lyophilized for 120 days.

Conclusion: It was possible to establish the upstream and downstream processing of LigANI fragment. The protein also demonstrated structural and thermal stability, which is a good indication for a potential vaccine candidate.

Keywords: process development; leptospirosis; recombinant protein