TITLE: INDUSTRIAL PRODUCTION DEVELOPMENT OF A NEW WHOLE CELL PERTUSSIS VACCINE WITH LOW REATOGENICITY

AUTHORS: AKAMATSU, M.A., CARVALHO B.P., SAKIHARA V.A, YANAI T.Y., TAKANO,I M.A.S., OLIVEIRA M.L.S., ABE C., NISHYAMA JR M.Y., CARVALHO E., MEROS, M., RAW, I., HO, P.L.

INSTITUTION: INSTITUTO BUTANTAN, SÃO PAULO, SP (AVENIDA VITAL BRASIL, 1500, CEP 0-900, SÃO PAULO – SP, BRAZIL).

ABSTRACT

The association of adverse events in immunization with Whole cell Pertussis Vaccine (wP) has led to the development of Pertussis Acellular Vaccine (aP). However, it is known that immunization with aP does not have the same efficiency compared to immunization with wP. Adverse reactions of wP are attributed to the presence of lipopolysaccharides (LPS). Our aim is to develop an industrial process of a new whole cell pertussis vaccine with reduced amount of LPS and low reatogenicity, while maintain the immunogenic properties, the whole cell Pertussis low vaccine (wPlow). 25 industrial lots were subjected to LPS extraction with organic solvent. Four possible industrial processes to separate the LPS from the cells were evaluated: tangential flow filtration (TFF), TFF with organic solvent wash (TFFSW), tubular centrifugation (CT) and continuous flow centrifugation (CFC), compared with wPlow produced at bench scale and with wP. wPlow were characterized by LPS content, endotoxic activity, cell integrity, presence of major vaccine antigens, immunogenicity and intranasal challenge in mice. The LPS reduction was 21%, 52%, 46% and 62% respectively using TFF, TFFSW, TC and CFC methods compared with wP. The endotoxin activity of pLow processed in a bench scale reduced 81% compared with a wP while the process using a TFF increase the endotoxin activity, and the TFFSW reduced in average 24%. The process with a TC reduced the endotoxin activity in 73% but the yield of this process was below 50%. The CFC decrease the endotoxin activity in 61% and the yield of this process was 62%. The cell integrity and major antigens were detected in all preparations. Based on these results, CFC were the choosen process. Anti-pertussis IgG were detected and results showed no differences in the B. pertussis colonization of lungs in mice immunized with wPlow or wP, indicating its potential as vaccine. The genome of the production strain was sequencing (genbank CP010323), the virulence factors polymorphism were identified and genes related to LPS biosynthesis/modifications were mapped, suggesting the presence of a pentacylated LPS. Modifications in pagP and pagL genes suggested a reduced endotoxic activity of LPS whereas the presence of genes involved in the addition of a glucosamine in the phosphate group of the Lipid A suggested an increase in TLR4-activity. The wPlow industrial production process was established, the perspective is to produce for clinical trials in the next few years.

KEYWORDS: Whole cell pertussis vaccine, LPS, Industrial process, *Bordetella pertussis*.

SUPPORTED BY: Fundação Butantan, BNDES, FAPESP and CNPq.