Evaluation of effect of pH on the yield conjugation of tetanus toxoid and Vi-capsular polysaccharide as a vaccine candidate for salmonella typhi

Background and aims: Typhoid fever is a systemic infection caused by Salmonella typhi. The disease remains an important public health problem in developing countries. The Vi-capsular polysaccharide of salmonella typhi (Vi-CPS), a licensed vaccine for typhoid fever is not suitable for routine immunization. To improve Vi-CPS immunogenicity Vi-covalently was bound to tetanus toxoid (TT) as a carrier.

Material & Methods: Standard strain of salmonella typhi Ty6s was grown under submerge cultural conditions in pilot-plant scale of 50 liter fermentor. Finally exponential growth phase, crude polysaccharide was obtained from fermentation broth by detergent-phenol extraction method and purified by ultracentrifuge differentiation technique. Vi-CPS was conjugated to TT by adipic acid dihydrazid (ADH) and 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDAC) method and Sepharos 4B-CL column chromatography. For increase yield of conjugation we use different pH from alkaline to acidic range in conjugation. The prepared antigens were injected intraperitoneally to BALB/c mice groups. Vaccination was performed by three doses with two week interval. Then serum samples was collected and antibody responses against Vi-CPS was measured by ELISA method for total IgG and IgG subclasses.

Results: Results of difference pH conjugation were shown that acidic pH better than alkaline pH and the best pH for increase yield of conjugation in this study was 6.4 and results of antibodies titers were shown that third inoculation of antigens for Vi-TT and Vi had enhances of antibody titers for all antibodies. Also Vi-TT was shown the highest titers in all classes of antibodies.

Discussion: Our results indicate that acidic pH better than alkaline pH and injection of covalently bound purified Vi-CPS to a T-dependent proteins elicited much higher levels of antibodies than Vi-cps alone besides, this conjugate it induce a booster responses in mice. Therefore this prepared conjugate is effective against salmonella typhi and candidate vaccine for this bacteria.

Key words: Salmonella typhi, Vi-TT conjugate, yield conjugation, tetanus toxoid.

Presentation: Poster