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First characterization of immunogenic conjugates of Vi negative *Salmonella typhi* O-specific polysaccharides with rEPA protein for vaccine development

Muhammad Salman[1, 2, 4], F St Michael[1], A Ali[2], A Jabbar[5], C Cairns[1], A C Hayes[1], M Rahman[2], M Iqbal[2], A Haque[3] and **A D Cox[1]**
[1]National Research Council, Canada
[2]National Institute for Biotechnology, Faisalabad, Pakistan
[3]University of Faisalabad, Pakistan
[4]Abasyn University, Pakistan
[5]Mirpur University of Science & Technology, Pakistan

Efficacious typhoid vaccines for young children will significantly reduce the disease burden in developing world. The Vi polysaccharide based conjugate vaccines (Vi-rEPA) against *Salmonella typhi* Vi positive strains has shown high efficacy but may be ineffective against Vi negative *S. typhi*. In this study, for the first time, we report the synthesis and evaluation of polysaccharide-protein conjugates of Vi negative *S. typhi* as potential vaccine candidates. Four different conjugates were synthesized using recombinant ExoProtein A of *Pseudomonas aeruginosa* (rEPA) and human serum albumin (HSA) as the carrier proteins, using either direct reductive amination or an intermediate linker molecule, adipic acid dihydrazide (ADH). Upon injection into mice, a significantly higher antibody titer was observed in mice administrated with conjugate-1 (OSP-HSA) (P=0.0001) and conjugate 2 (OSP-rEPA) (P≤0.0001) as compared to OSP alone. In contrast, the antibody titer elicited by conjugate 3 (OSPADH-HSA) and conjugate 4 (OSPADH-rEPA) were insignificant (P=0.1684 and P=0.3794, respectively). We conclude that reductive amination is the superior method to prepare the *S. typhi* OSP glycoconjugate. Moreover, rEPA was a better carrier protein than HSA. Thus, OSP-rEPA conjugate seems to be efficacious typhoid vaccines candidate, it may be evaluated further and recommended for the clinical trials.

s.amazai@yahoo.com