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

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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 exo-protein 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) ($P\leq0.0001$) 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 glycol-conjugate. 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.

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