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Shigella sonnei oligosaccharide-protein conjugates

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Abstract

Synthetic oligosaccharides composed of several repeats of *Shigella dysenteriae* type 1 O-specific polysaccharide (O-SP), bound by their reducing ends to a carrier protein ("sun" type configuration), induced in mice significantly higher antibody levels than conjugates of the native O-SP ("lattice" type configuration). Here we present isolation and characterization of low molecular mass oligosaccharides of *Shigella sonnei* lipopolysaccharides and their conjugation to several carrier proteins. Conjugates were formed by oxime linkages between the terminal Kdo residues of the reducing ends of the saccharides and aminooxy linkers bound to the protein. IgG antibody levels induced in young outbread mice by these conjugates were significantly higher than those prepared with the full length native O-SP. We propose clinical evaluation of the new *S. sonnei* conjugates.

Keywords: shigell;, *sonnei*; LPS; conjugate; vaccine;

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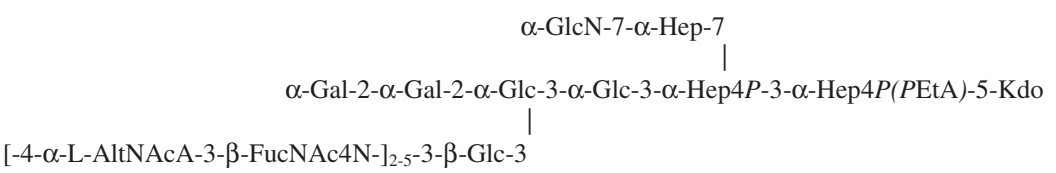
Shigellae cause endemic and epidemic diarrhea and/or dysentery worldwide, especially in developing countries. At least one half the cases and deaths occur in children less than 5 years old. Despite its discovery over a century ago, there is still no licensed vaccine for shigellae. Residual morbidity, even after effective antibiotic therapy, and increasing antibiotic resistance of shigellae urge the development of vaccines to prevent disease caused by this pathogen.

Lipopolysaccharides (LPS) of *Shigella* are essential virulence factors. An LPS-based vaccine elicited 74 % protection against shigellosis in Israeli Army recruits at 8-14% infection rates [1]. The efficacy was related to vaccine induced IgG anti-O-SP levels. Evaluation of such conjugates in children, having highest incidence and severity of *S. sonnei* shigellosis, showed an age related antibody responses and protection.

A significant improvement in the immunogenicity of *S. dysenteriae* type 1 conjugates was achieved using short synthetic fragments of O-SP bound by their reducing ends to a protein carrier [2]. However the synthesis of *S. sonnei* O-SP oligosaccharides has not been possible to date. We used therefore low mass O-SP-core (O-SPC) fragments isolated from the LPS to bind to carrier proteins, similarly to the preparation of the synthetic *S. dysenteriae* type 1 oligosaccharide-protein conjugates.

LPS from *S. sonnei* strain 53G was extracted by the hot phenol method and purified by ultracentrifugation. Next, LPS was hydrolyzed with 1% acetic acid at 100°C for 1.5 h, Lipid A removed by ultracentrifugation and the soluble product subjected to gel chromatography on a BioGel P-10 (1x100 cm) column in pyridine/acetic acid/water buffer (4/8/988 ml). Four fractions were obtained and analyzed by NMR and mass spectroscopy [1]. Integration of the O-SP FucNAc4N methyl signal in ^{1}H-NMR spectra (1.34-1.36 ppm) relative to the anomeric signals of core α-Gal M (5.82 ppm) and α-Gal L (5.62 ppm) showed that fraction F1 contained core with approximately 29 O-SP repeat units (RU), F2: core with an average of 3.5 RU, and F3: core with an average of 1.3 RU. Fraction F4 contained various degradation products. Fraction F2 (O-SPC-F2) was analyzed in detail and the following structure was assigned:



Four conjugates were prepared by binding O-SPC-F2 to either BSA (conj. **1**; BSA/O-SPC-F2) or to recombinant diphtheria toxin (conj. **2, 3** and **4**: rDT/O-SPC-F2). The conjugation was based on the formation of stable oxime linkages between the Kdo residue present at the O-SPC reducing-end and an aminooxy linker bound to the carrier protein [2, 3]. Conjugates were analyzed by MALDI-TOF mass spectrometry, SDS-PAGE and protein and sugar colorimetric assays; all methods provided comparable

of the carrier protein and of the O-SPC. Characterization of conjugates is presented in Table 1. All conjugates reacted by double immunodiffusion with rabbit anti-*S. sonnei* and anti-protein sera by an identity line.

All conjugates elicited low levels of IgG anti-LPS after the 2nd injection with booster responses after the 3rd. The geometric means (GM) of IgG anti-LPS are presented in Table 1. There were no statistical differences after the 3rd injection between conjugates **1**, **2** and **4**, which contained average 6-7 O-SPC-F2 chains per protein molecule. Conjugate **3,** which contained twice as much of O-SPC-F2 chains per rDT than the other conjugates, induced statistically lower antibody levels both to the saccharide and the protein. All four O-SPC-F2 conjugates induced statistically higher antibody levels then the 'lattice' type conjugate prepared with the full length O-SP.

Table 1. Composition and GM of serum IgG anti-*S. sonnei* LPS induced by O-SPC-F2 conjugates bound to bovine serum albumin (BSA) or to recombinant diphtheria toxin (rDT) and by full length O-SP bound to recombinant *P. aeruginosa* Exotoxin A (rEPA).

No	Conjugate	Mm of conjugate [kDa]	Mm of sugar part [kDa]	No. of O-SPC chains per BSA or rDT molecule	IgG [EU] anti-LPS		IgG [EU[1]] anti-rDT	
					2nd inj.	3rd inj.	2nd inj.	3rd inj.
1	BSA/O-SPC-F2[2]	93.1	22.2	7	79	366	nd[3]	nd
2	rDT/O-SPC-F2	80.6	18.5	6	5	392	2	91
3	rDT/O-SPC-F2	99.5	37.4	12	11	150	0.1	2
4	rDT/O-SPC-F2	81.1	19.0	6	19	328	1	11
5	rEPA/O-SP[4]	nd	nd	nd	11	67	nd	nd

[1]EU = ELISA Units; [2]*S. sonnei* O-SPC-F2 = core + avr. 3.5 repeat units (RU) of O-SP; avr.molecular mass (Mm) = 3206 Da; [3]nd = not determined;[4]*S. sonnei* O-SP = core + avr. 29 RU of O-SP bound by multi-point attachment

Mice (10 per group) were injected with 2.5 μg of saccharide as a conjugate per mouse, 3 times, 2 weeks apart and bled one week after the last two injections.

Conclusion

Protein conjugates of the low molecular mass oligosaccharides containing LPS core plus average 2-5 O-SP repeat units (O-SPC) were prepared and characterized by physico-chemical and immunogenicity assays. These conjugates induced significantly higher IgG antibody levels then those

method is being studied with *S. flexneri* 2a, 6 and *S. dysenteriae* type 1 LPS. The mild reaction conditions and relative ease of preparation provided by O-SPC may be applicable for the preparation of conjugates against other Gram-negative pathogens such as non-typhoidal *Salmonella* or *Escherichia coli* O157.

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