

V7 - Characterization of oligosaccharide of *Neisseria meningitidis* Serogroup B

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Introduction:

The Brazilian vaccine against meningococcal disease *Neisseria meningitidis* serogroup B is composed by outer membrane vesicles (OMV) and chemically modified endotoxin (dLOS); from prevalent strains in Brazil. The use of chemically modified endotoxin aims to improve the efficiency of the vaccine. Studies on the biosynthesis of LOS from *Neisseria meningitidis*, indicates the existence of 12 different immunotypes (L1 to L12). Their structural characterization is relevant for studies related to vaccine development. Lipooligosaccharides are composed by two distinct regions, the lipid that is connected to the central part of the molecule and the oligosaccharide core (OS), which is divided into an inner and outer region. The inner region is comprised of 3- deoxy-D-manno-ulosonic acid and L-glycero-D-manno heptoses (Hep). The outer region extends further from the bacterial surface and it is composed predominately by hexoses and hexosamines. According to the literature, bactericidal activity of induced antibody can be increased by oligosaccharide core of LOS.

Objective:

The aim of this study is to perform the structural characterization of oligosaccharides by different analytical approaches as mass spectrometry (NMR 1H, gCOSY, TOCSY, ROESY, HSQC and HMQC 1H x 31P), CGAR, and CE-MS, to evaluate if it is preserved after chemical modification.

Methodology:

The OS was obtained by acid hydrolysis of LOS lyophilized and purified by exclusion chromatography using Biogel P-4 as stationary phase. The samples were decomposed by methanolysis, silanized and analyzed by GCMS (IE, 70 eV) to determinate the glycoside composition. The molecular weight and the core saccharide sequence were determined by CE-ESI/MSn. The NMR

(^1H NMR, gCOSY, TOCSY, ROESY, HSQC and HMBC) data were acquired on 500 MHz spectrometer, using deuterium oxide, as solvent, with acetone as internal reference.

Results:

The glycosidic composition comprises of galactose, N-acetylglucosamine, glucose; L-glycero-Dmanno-heptose; 3-deoxy-D-manno-ulosonic The CE-ESI/MSn analysis detected an ion at $[\text{M1} + \text{H}] + 1639.5$ its molecular weight and fragmentation pattern was related to L-7. The analysis of NMR data it was possible assign the hydrogens and carbon constituents of the OS structure and to establish, through the ROESY data the connectivity across glycosidic linkage. The. $^1\text{H} \times 3 \text{ 1 P}$ HMQC showed a phosphorylation at position (3) in heptose structure.

Conclusion:

In this study, we conclude that the major immunotype of the vaccinal LOS is L7 and that the core does not suffer alteration during the chemical detoxification stage.

Keywords: Oligosaccharide, *Neisseria meningitidis*.