

Physicochemical and immunological characterization of the Drug Product (final lot) of a meningococcal group A conjugate vaccine.

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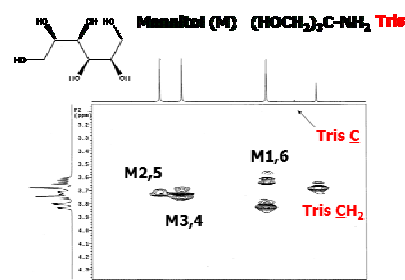


1. INTRODUCTION

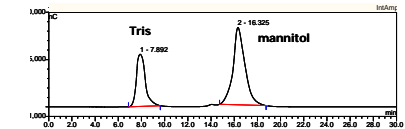
The meningococcal group A organism is responsible for 90% of the cases of endemic and epidemic meningitis caused by *Neisseria meningitidis* bacteria in the Meningitis Belt of sub-Saharan Africa. Preventive immunisation should avoid a great number of deaths and be less expensive than mass immunisation campaigns performed with polysaccharide-only vaccines after epidemics have begun. This is best achieved by vaccination with conjugate vaccines which, unlike polysaccharide vaccines, are immunogenic in the very young, induce immunological memory and are likely to give long-lasting protection. MVP (Meningitis Vaccine Project [1]) is developing an affordable monovalent meningococcal A conjugate vaccine for sub-Saharan Africa which is manufactured by Serum Institute of India Limited (SIIL) using aldehyde-hydrazide condensation chemistry developed at the US Center for Biologics Evaluation and Research [2]. The labile nature of the PsA phosphodiester linkage means that special attention needs to be paid to the integrity and stability of the conjugate vaccine. Typically stability-indicating assays involving size analysis and free saccharide determination are performed on the bulk conjugate. In keeping with the increasing emphasis being placed on the quality of the vaccine administered, the WHO Recommendations for the group A conjugate vaccines includes these assays on the final lot [3]. For a lyophilized final product, the presence of large amounts of excipients means that appropriate methods need to be developed for the full physicochemical characterization and testing of the Drug Product. Preclinical analysis of the final product has also been included.

3. EXCIPIENTS BY NMR AND HPAEC-PAD

¹H-¹³C HSQC map of final lot PsA-TT (4 vials)



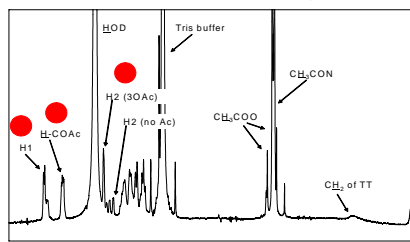
HPAEC-PAD analysis of excipients



NMR established the structure and identity of the mannitol and tris excipients and indicated their relative proportions, which was confirmed by HPAEC-PAD analysis.

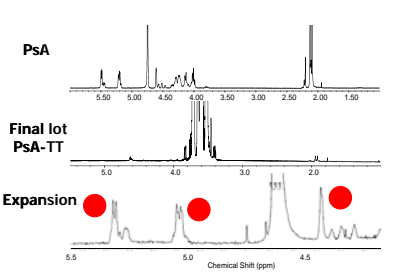
4. PsA-TT STRUCTURE BY NMR

¹H NMR spectrum of bulk conjugate PsA-TT



Contains characteristic signals for O-acetylated PsA

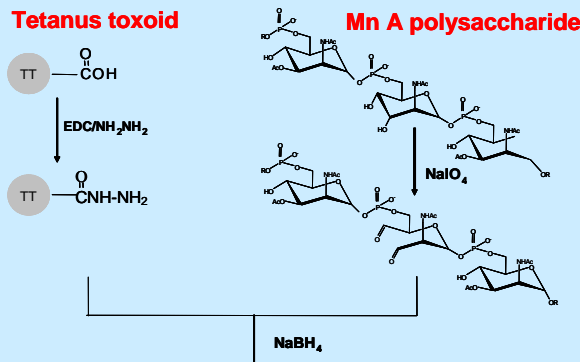
¹H NMR spectrum of final lot PsA-TT



At 600 MHz, expansion of the anomeric region showed the similarity of the O-acetylation pattern to that of PsA, thus confirming the structural integrity of the saccharide in PsA-TT

NMR spectra were recorded on the final fill dissolved in D₂O using a Varian Unity 400 (University of Cape Town) or Varian Inova 600 (University of Stellenbosch) NMR spectrometer. The 2D ¹H/¹³C heteronuclear correlation (HSQC) experiment was optimized for J = 140 Hz. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was performed on a Dionex DX-500 system using a CarboPac PA1 or MA1 column and a NaOH/NaOAc elution gradient.

2. CONJUGATION PROCESS and CONTROL



Mn A-TT conjugate vaccine

Formulated with mannitol/tris buffer and lyophilized, 120 µg PsA/vial

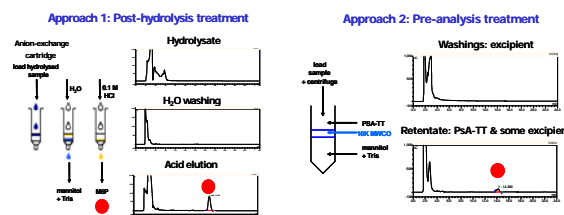


WHO Recommendations for Mn A: control tests on final lot

1. Identity
2. Sterility
3. Saccharide content
4. Conjugated v. free saccharide
5. Molecular size distribution
6. Residual moisture
7. Pyrogen content
8. Adjuvant content (if used)
9. Preservative content (if used)
10. General safety test
11. pH
12. Inspection

Further characterization of the final lot may be performed by use of physicochemical techniques and animal studies.

7. ONGOING RESEARCH-2



Both methods show potential for free saccharide determination on final fill samples.

8. CONCLUSIONS

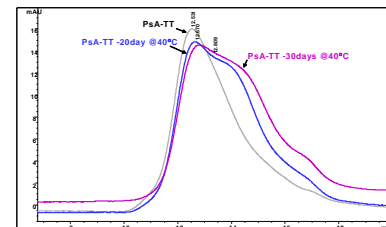
Physicochemical methods have been applied to characterize the final lot of a meningococcal A conjugate. NMR spectroscopy identified and quantified the excipients present and use of a high field instrument permitted confirmation of the structural integrity of the PsA component in the final lot. The size distribution of PsA-TT was profiled by SEC-HPLC which also indicated the stability of PsA-TT at 40°C. The free saccharide assay was shown to be a sensitive indicator of conjugate integrity and showed that the final fill met specifications after storage for two years at 2-8°C. HPAEC-PAD is a sensitive technique for PsA quantification, two approaches for minimizing interference from excipients were presented. The physicochemical characterization and immunogenicity in mice confirmed the quality of the PsA-TT vaccine; this accords with the clinical trial results reported elsewhere.

Clinical trials posters (P211, P134, P216, P223 and P227)

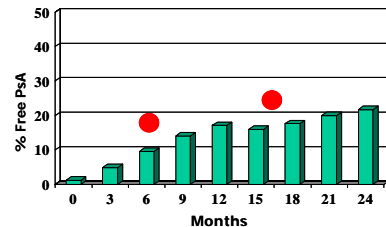
9. REFERENCES

5. SIZE/STABILITY OF PsA-TT

SEC-HPLC-UV: PsA-TT size distribution at 40°C



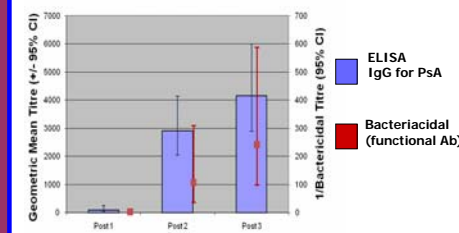
Stability study of final lot at 2-8°C



Clinical trials- good immunogenicity and immunological memory in toddlers (P211)

SEC-HPLC indicated the integrity of PsA-TT; samples stored at 40°C showed peak broadening and the formation of lower MWt peak. The free saccharide assay was shown to be a sensitive indicator of conjugate integrity; the DOC/HCl/phosphate method was validated. The final fill was shown to meet specifications after storage for two years at 2-8°C and to be immunogenic in toddlers.

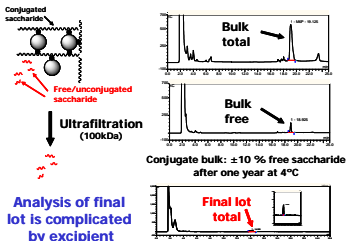
6. IMMUNOGENICITY IN MICE



Antibody (Ab) titres increased significantly (P<0.05) following a second dose of vaccine, but not significantly after a third dose. There was a partial correlation (r>0.6) between total IgG and functional Ab at post-dose 2 & 3.

7. ONGOING RESEARCH-1

Total and free saccharide by HPAEC-PAD



High performance size-exclusion chromatography (SEC-HPLC) was performed with ultra-violet (UV) detection on an Agilent 1200 instrument. PsA content was determined using the phosphate assay or following hydrolysis and quantification of mannosamine-6-phosphate by HPAEC-PAD. Free/unbound saccharide was separated from conjugate by use of deoxycholate/acid (DOC/HCl) precipitation or by ultrafiltration. Immunogenicity studies were performed in mice immunized with 3 doses of vaccine (1 µg PsA/dose); total PsA-specific IgG and functional bactericidal activity (SBA) of sera were determined.

[1] MVP is a partnership between WHO and PATH with the goal of eliminating epidemic meningitis (group A) from sub-Saharan Africa through the development, testing, licensure and widespread use of conjugate meningococcal vaccines (www.meningitis.org) [2] C. E. Frasch, S. Kapre, S. Beri, D. M. Granoff, N. Bouwer, F. M. LaForce, F. M. C. H. R. Lee, A novel conjugation process for production of a highly immunogenic Group A meningococcal conjugate vaccine for use in Africa. Abstract, 14th International Pathogenic Neisseria Conference, Milwaukee, Wisconsin, USA, September 5-10, 2004. [3] WHO Recommendations to assure the quality, safety and efficacy of group A meningococcal conjugate vaccines, approved by the Expert Committee on Biological Standardization (ECBS) in October, 2006 (<http://www.who.int/biologicals/publications/trs/areas/vaccines/meningococcal/en/index.html>)