Poster 17

THE GLYCOSYLATION PATTERN OF FELINE ALPHA 1 ACID GLYCOPROTEIN (AGP)

Cunningham K[1], Smith KD[2], Addie DD[3], Eckersall PD[1]
[1]Division of Animal Production & Public Health, University of Glasgow, Glasgow, Scotland [2]Department of Bioscience, University of Strathclyde, Glasgow, Scotland. [3]Division of Infection and Immunity, University of Glasgow, Glasgow, Scotland. E mail: k.cunningham@vet.gla.ac.uk

Introduction

Alpha 1 acid glycoprotein is a positive acute phase protein in humans and cats as its plasma concentration increases 2-5 fold during the acute phase response. Alpha 1 acid glycoprotein is extensively glycosylated with 5 complex type N-linked glycan chains that account for 45% of its 41-44 kDa molecular weight. In normal plasma AGP does not exist in a single form but as heterogeneous population due to subtle changes in the N-linked oligosaccharide chains. Since the oligosaccharide chains attached influence the functional role of AGP, the existence of structural distinct glycoforms in plasma implies functional diversity. During several physiological and pathological conditions in man, not only the total concentration of AGP is altered but the relative proportions of the normal AGP glycoforms have also been found to change and abnormal glycoforms are expressed with the oligosaccharide "fingerprint" of AGP being altered. The plasma concentration of AGP is known to be increased in feline infectious peritonitis (FIP) and is now used as part of a panel of tests to diagnose FIP. At present there is relatively little information on the diagnostic significance of AGP glycosylation in this diseases.

Materials and Methods

The concentration of AGP was measured in peritoneal fluid and plasma submitted to the feline virus diagnostic laboratory of Glasgow Veterinary School using radial immunodiffusion. Thereafter AGP was purified from the remnant of each sample after all diagnostic tests were complete using a method which ensured that no desialylation of the oligosaccharide chains or denaturation of the protein structure occurred. The samples were initially precipitated with 30 % w/v Polyethylene glycol (PEG) Mwt 8000, followed by three chromatography steps, which utilized affinity, anion and cation exchange chromatography resins and desalting. After purification samples were either hydrolysed with acid or digested with PNGase F which releases the oligosacchrides. After acid hydrolysis the monosaccharide composition was determined using High pH Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). Samples digested with PNGase F were also analysed using HPAEC-PAD.

Results

Initial analysis of feline AGP revealed differences in glycosylation between healthy and diseased cats. Fucose, Mannose, Galactose and Glucosamine were found on feline AGP from FIP infected cats however little or no fucose was found on normal feline AGP. Oligosaccharide analysis revealed that feline AGP purified from peritoneal fluid of FIP infected cats had mainly bisialylated biantennary glycan chains attached to the polypeptide backbone.

Conclusion

The glycosylation pattern of feline AGP has novel features and could be diagnostic for the appearance of FIP, opening up possibilities for treatment.