

INVESTIGATION OF SULPHATED OLIGOSACCHARIDES AS MARKERS OF HEPARAN SULPHATE ACCUMULATION IN MURINE MUCOPOLYSACCHARIDOSIS TYPE IIIA USING ESI-MS/MS

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Mucopolysaccharidosis type IIIA (MPS IIIA), one of more than 45 inherited lysosomal storage disorders occurs as a result of blocked heparan sulphate (HS) catabolism. HS catabolism begins with endo-degradation of the long chain polymer to smaller HS oligosaccharides, followed by the sequential action of lysosomal exoenzymes to reduce these oligosaccharides to monosaccharides and inorganic sulphate. MPS IIIA is characterised by a deficiency of the exoenzyme sulphamidase. An inability to hydrolyse non-reducing end glucosamine N-sulphate esters leads to the accumulation of partially degraded HS oligosaccharides in lysosomes of affected cells; progressive deterioration of cells, tissues and organs; and the excretion of HS oligosaccharides in the urine.

We isolated partially degraded HS fragments from the urine of an MPS IIIA patient using anion exchange and gel filtration chromatography. Di- to hexadecasaccharides were characterised using electrospray ionisation mass spectrometry in negative ion mode. These oligosaccharides were shown to have non-reducing N-sulphated glucosamine residues susceptible to digestion with sulphamidase. These oligosaccharides were not present in normal control urine and as such may be useful biomarkers for MPS IIIA. To this end we determined relative levels of di- to hexasaccharides by high performance liquid chromatography electrospray ionisation-tandem mass spectrometry in the brain, spleen, lung, heart, liver, kidney and urine of a naturally occurring mouse model of MPS IIIA. All MPS IIIA mouse tissues showed an increased level of HS storage compared to control mice, although there was considerable difference between the level of storage in different tissues. This difference in HS storage levels was also reflected in the levels of oligosaccharides present in each tissue type. The different relationships observed between oligosaccharides and total UA may indicate that endodegradation of HS varies between tissues. In light of this it may be appropriate to define the relationship between oligosaccharide markers and HS storage for each tissue studied. Oligosaccharides may be useful to evaluate the effects of therapeutic strategies, including gene, enzyme replacement and substrate deprivation therapy, in the mouse model of MPS IIIA. We anticipate that changes to oligosaccharide levels will reflect correction of pathology in tissues following therapy.