

Monitoring Oligosaccharides in Urine, Plasma and Blood Samples from Mucopolysaccharidoses Patients by Electrospray Ionisation Mass Spectrometry

Steven L. Ramsay, Tina Rozaklis, John J. Hopwood, Peter J. Meikle

Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia

The mucopolysaccharidosis (MPS) group of disorders is characterised by the lysosomal storage of glycosaminoglycans (GAGs) that results from an enzyme deficiency in any one of nine GAG degrading enzymes resulting in elevated urine, plasma and blood concentrations. The lysosomal MPS subgroup of genetically inherited metabolic disorders has a collective incidence in Australia of approximately 1 in 16,000¹. This frequency is significantly high in neonatal births for the implementation of a neonatal screening program being pursued by this department and could presymptomatically disseminate between affected neonates from normals an immense advantage for clinical treatment.

Levels of sulfated N-acetylhexosamine monosaccharides have been determined in human urine, plasma and dried blood spots. Samples were derivatised with phenyl 3-methyl pyrazolone followed by C18 solid phase extraction column chromatography prior to analysis by electrospray mass spectrometry. Among the many putative PMP derivatised oligosaccharides observed the monosulfated N-acetylhexosamine (HexNAcS) monosaccharides were most prominent. Indicative of the mucopolysaccharidosis (MPS) disease states, most patient samples showed significantly increased HexNAcS levels in urine² blood and plasma using a deuterated GlcNAc6S-*d*₃ internal standard. Concentrations of the total HexNAcS analytes showed a strong correlation with disease severity in MPS particularly MPS-IIID, -IVA and -VI. Urine from MPS-IIID, -IVA and -VI showed the largest elevations above the mean of the control value (17, 47 and 75 fold respectively). These disorders showed large elevations in other monitored oligosaccharides, an N-acetylgalactosamine 4,6-disulfate (GalNAc4,6S) and a disaccharide with proposed structure N-acetylgalactosamine 4/6-sulfate-(1→4)-O-D-glucuronic acid (GalNAc4/6S(β1-4)GlcA). A retrospective study of bone marrow transplanted MPS-IVA and MPS-VI patients both showed a decrease in the urine concentration of HexNAcS, GalNAc4,6S and GalNAc4/6S(β1-4)GlcA that correlated with clinical improvement. These metabolic markers identified this far have potential applications in diagnosis, phenotype prediction and monitoring of current and future therapies for the MPS IIID, IVA and VI disorders.

1. Meikle, P.J., *et al.*, Prevalence of lysosomal storage disorders. JAMA, 1999. **281**(3): p. 249-54.
 2. Hopwood, J.J. and H. Elliott, Urinary excretion of sulphated N-acetylhexosamines in patients with various mucopolysaccharidoses. Biochem J, 1985. **229**(3): p. 579-86.
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