ENZYME REPLACEMENT THERAPY
IN A MURINE MODEL OF
MUCOPOLYSACCHARIDOSIS
TYPE IIIA

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Thesis submitted for the degree of
Doctor of Philosophy
in
The University of Adelaide
(Faculty of Medicine)

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December 2002
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Macropoly saccharidosis type III A (MPS IIIA, Sanfilippo A syndrome) is an autosomal recessive lysosomal storage disease, with a prevalence in Australia of 1 in 114,000. MPS IIIA is caused by a deficiency of the lysosomal enzyme sulfamidase which is needed together with other exohydrolases and a N-acetylgulansomerase to break down the glycosaminoglycan heparan sulphate to sulphate and monosaccharidases. Patients are characterised by severe central nervous system (CNS) degeneration together with mild somatic involvement; this disproportionate correlation is unique amongst the mucopolysaccharidoses (MPS). Features include severe behaviour disturbances, such as hyperactivity and aggressiveness, coarse hair and mild hepatosplenomegaly. Death is usually in the mid- to late-teenage years. Enzyme replacement therapy (ERT) by intravenous administration of recombinant human NS (rhNS) has been proposed as a potential therapy for MPS IIIA.

A naturally occurring mouse model for MPS IIIA has been described and a colony of these mice has been established. The mice exhibit many of the debilitating features found in human MPS IIIA patients. The MPS IIIA mouse provides an excellent model for developing and testing treatment strategies.

The mutation causing MPS IIIA in the mice was identified as a base substitution at codon 31 in the sulfamidase gene, altering an aspartic acid to an asparagine (D31N). This aspartic 31 (D31) is involved in binding of the divalent metal ion needed for the catalytic function. When overexpressed in CHO-K1 cells the intracellular D31N sulfamidase protein corresponded to 1.5% of wild-type sulfamidase levels as determined using a radio labelled tetrasccharide substrate. Recombinant murine sulfamidase (mNS) was expressed in CHO-K1 cells to levels of 4 mg/L of culture medium and purified to apparent homogeneity using a two-step ion exchange method. Denaturing and reducing SDS/PAGE revealed a subunit size of 62 kDa. Kinetic analysis demonstrated that mNS had kinetic parameters similar to rhNS and human sulfamidase purified from tissue. rhNS could be taken up by MPS IIIA skin fibroblasts via a mannose-6-phosphate receptor mediated process.
Before therapy trials were initiated MPS IIA mice were tested to assess cognitive functions. The Morris water maze (MWM) assesses spatial learning abilities in rodents. The MWM was performed on MPS IIA and normal control mice at various ages throughout development. At seven-weeks of age no significant difference was observed between MPS IIA and normal mice. At 15-weeks of age MPS IIA mice performed notably worse than normal mice, suggesting that neurological deterioration had commenced. By 20-weeks of age a statistically significant difference was observed between the MPS IIA and normal mice, with the MPS IIA mice performing significantly worse than the normal mice in every phase of the testing. From this study it was concluded that severe neurological deterioration of the MPS IIA mice, as measured in the MWM, occurs by 20-weeks of age.

For many inborn errors of metabolism, early treatment is vital in preventing long-term clinical pathology. The major site of pathology in the MPS IIA mouse is the CNS. Therefore therapy protocols must address the problem of being able to target sufficient amounts of enzyme to the CNS in order to reverse or prevent the cognitive deficits associated with the disease. Transport of molecules from the blood into the CNS is greatly restricted due to the presence of very tight junctions between the endothelial cells of the cerebrovascular capillary walls, known as the blood-brain barrier (BBB). Mice are born with an incompletely formed BBB, which is not fully intact until 10- to 14-days of life. Enzyme distribution studies, by intravenous administration of 1 mg/kg of rhNS were performed on MPS IIA mice on day one of life and at six-weeks of age. Enzyme was detected in the CNS of mice who received enzyme from birth, but not in mice injected with enzyme at six-weeks of age.

Long-term ERT was performed on MPS IIA mice, initiated either at birth or at six-weeks of age. MPS IIA and normal control mice received weekly intravenous injections with 1 mg/kg of rhNS until 20-weeks of age. Treatment initiated at birth was more effective than treatment initiated in young adults. In general male MPS IIA mice housed together become aggressive and fight by 10-weeks of age. MPS IIA mice who began treatment at six-weeks also began fighting at 10-weeks of age whereas male MPS IIA mice, treated from birth were not aggressive until at least 18-weeks of age. Throughout the 20-week study, control mice did not show this aggressive behaviour. Neurological function was measured using the MWM. MPS IIA mice, treated from birth, and normal
mice performed equally and significantly better, in every phase of the test, than MPS IIIA mice treated from six-weeks of age or left untreated. Histology showed no storage vacuoles in neurons of the cerebellum and cerebral cortex in three-week MPS IIIA mice treated from birth. By eight-weeks of age minimal storage vacuoles had started to return to these cells. Open membranous storage vacuoles were present in age matched MPS IIIA mice, untreated or treated from six-weeks of age. By 14-weeks of age the neurons in MPS IIIA mice, untreated or treated from six-weeks of age contained abundant storage vacuoles, which were membranous whorls and stacks, a pattern also observed at 18- and 23- weeks of age. In contrast by 14-weeks of age open membranous storage vacuoles were present in MPS IIIA mice enzyme treated from birth; it was not until 18-weeks of age that membranous whorls and stacks were observed in the neurons of these mice. These experiments suggest that rhNS, entering the brain in the first few weeks of life, before the BBB matures, is able to retard the development of behaviour and learning difficulties in MPS IIIA mice.