ENZYME REPLACEMENT THERAPY
IN A FELINE MODEL OF MUCOPOLYSACCHARIDOSIS TYPE VI

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THESIS ABSTRACT

Mucopolysaccharidosis Type VI (MPS VI) is an inherited lysosomal storage disorder, due to a deficiency of N-acetylgalactosamine-4-sulphatase (4S), leading to an accumulation of dermatan sulphate (DS) in many tissues. Severely affected human patients exhibit severe skeletal abnormalities causing dwarfism and facial dysmorphia, as well as widespread soft tissue pathology. Death is usually in late childhood. Bone marrow transplantation is currently the only therapy available for these patients, and has alleviated some problems, however improved outcomes by new therapies are needed. Enzyme replacement therapy (ERT) by intravenous administration of artificially produced recombinant human 4S (rh4S) has been proposed as an alternative therapy for MPS VI.

MPS VI has also been described in Siamese cats, and a colony related to these original cats has been established. Disease in the feline model is similar to that in humans. The primary aim of this study was to evaluate the efficacy of ERT with rh4S in feline MPS VI and to test the hypothesis that this form of therapy would reverse or alter the disease course, particularly the bone dysplasia and connective tissue pathologies. Preliminary ERT studies at low enzyme doses in young and adult MPS VI cats suggested that the earlier therapy began, the greater the improvement in bone histomorphometric parameters, although ERT was unable to alter the overall progression of disease. In enzyme distribution studies in normal cats, the majority of enzyme was detected in the liver, however enzyme was also detected in most other tissues except cartilage and cornea.

Subsequent ERT was started at birth at various dose rates. Some cats were treated with rh4S coupled to ethylene diamine or poly-L-lysine to improve penetration of enzyme into cartilage. Efficacy of ERT was evaluated by: clinical examination, radiographs, bone histomorphometry, quantitation of urinary glycosaminoglycans and tissue histology. Plasma antibody titres against rh4S were also measured. ERT resulted in subjective improvements in mobility, overall size and appearance of treated cats. Increased bone lengths and more uniform bone density was observed radiographically, corresponding with improvements in bone histomorphometric parameters. A greatly reduced incidence of spinal cord compression and a dose dependent reduction in urine glycosaminoglycan excretion was also observed. Variable reduction of storage vacuoles was observed in most connective tissues,
including heart valve, however chondrocytes and corneal keratocytes remained unchanged at all doses. Response to therapy was dose dependent, with improved response seen at higher doses. Significant joint pathology was also present in MPS VI cats with or without ERT at 11 months of age. Only one MPS VI cat undergoing intravenous ERT from birth had titres elevated above those in untreated MPS VI and normal control cats.

The original mutation causing the MPS VI phenotype (L476P) was identified during this project, providing a rapid PCR based identification of heterozygotes, which improved breeding within the colony. Subsequent detection of a second mutation (D520N), led to the identification of six genotypes within the same colony, and additional abnormal phenotypes. L476P/L476P cats used in ERT studies exhibit dwarfism, very low leukocyte 4S/β-hexosaminidase ratios, DSuria, corneal clouding, degenerative joint disease, and lysosomal inclusions in leukocytes and in most connective tissues including chondrocytes. D520N/D520N and L476P/D520N cats have similar biochemical features but have normal growth, lack corneal clouding, and only have inclusions in leukocytes, and some chondrocytes. L476P/D520N cats also have a high incidence of degenerative joint disease. We conclude that L476P/D520N cats have a very mild MPS VI phenotype distinct from the L476P/L476P phenotype.

Prevention or reduction of development of lysosomal storage in the majority of tissues at 1 and 5 mg/kg ERT from birth, suggests that ERT is likely to lead to significant improvements in quality of life in human patients. Improvements in bone growth and structure, and minimal or no lysosomal storage in heart valve, indicates that ERT can target important sites of pathology and alter the disease course. Our studies suggest that the greatest effect of ERT on skeletal disease may occur the earlier the age of onset of therapy. Despite attempts to improve cellular uptake by enzyme modifications, lysosomal storage was still present in cornea and cartilage, which are both avascular tissues. Further study of L476P/D520N and D520N/D520N genotypes will assist in understanding the pathogenesis of joint disease in MPS VI, and may aid in development of therapies to target this site of pathology. They will also improve understanding of genotype to phenotype correlations and the pathogenesis of skeletal dysplasia in MPS VI. These studies have also demonstrated that the feline model of MPS VI is a useful model for evaluation of new therapies.