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Repeated intrathecal injections of recombinant human 4-sulphatase remove dural storage in mature mucopolysaccharidosis VI cats primed with a short-course tolerisation regimen

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ABSTRACT

All MPS-VI cats treated thus far with weekly intravenous enzyme replacement therapy (IV ERT) with recombinant human *N*-acetylgalactosamine-4-sulphatase (rhASB) from 3 months of age onwards developed circulating anti-rhASB antibodies. In view of this, the possibility of inducing immune tolerance by using a short-course tolerisation regimen was tested. Starting at 4 months of age, MPS-VI ($n = 5$) and unaffected cats ($n = 2$) received cyclosporine and azathioprine over a 22-day period plus weekly IV ERT with 0.1 mg/kg rhASB. After a 4-week resting period, these cats were administered weekly IV ERT with 1 mg/kg rhASB until 11 or 17 months of age. Four unaffected cats ($n = 4$) received weekly IV ERT only. Health, growth and seroconversion were regularly monitored. Four out of five MPS-VI cats tolerated rhASB well, as indicated by negligible or low antibody titres and absence of hypersensitivity reactions. One MPS-VI cat exhibited elevated antibody titres and hypersensitivity reactions during some IV treatments. The two unaffected cats that received the tolerisation regimen remained seronegative, however, only half of the unaffected cats not submitted to this regimen seroconverted. Only minor side-effects were attributed to the short-course of cyclosporine and azathioprine. Two MPS-VI cats also well-tolerated four weekly intrathecal injections of rhASB and consequently exhibited less oligosaccharide fragments in cerebrospinal fluid and less vacuolation within their dura mater. These data indicate that a relatively high rate of immunotolerance towards rhASB can be achieved in MPS-VI cats with a short-course tolerisation regimen ultimately permitting removal of lysosomal storage within the dura mater with the use of intrathecal therapy.

Introduction

Mucopolysaccharidosis VI (MPS-VI or Maroteaux–Lamy syndrome) is an inherited lysosomal storage disorder (LSD) resulting from a mutation within the gene encoding for *N*-acetylgalactosamine-4-sulphatase (ASB or EC 3.1.6.1), which leads to the decrease or loss of functional enzyme activity. As a consequence, dermatan sulphate and chondroitin sulphate accumulate within the lysosomes of the connective tissue cells within most organs [1]. If a compatible donor is available, bone marrow transplantation can be considered as a therapeutic option for affected children [2–4], however, evidence now indicates that intravenous enzyme replacement therapy (IV ERT) with recombinant human 4-sulphatase (rhASB) can be a safer alternative offering short-term and longer-term benefits [5–10].

A naturally occurring feline model of MPS-VI has proven invaluable in the development and testing of treatment strategies for MPS-VI [11–14]. Indeed, in MPS-VI cats, weekly IV ERT with rhASB from birth has been extremely effective in reducing the majority of pathology associated with the disease [11,12]. For example, pathology in heart valve, aorta, bone, liver and kidney is reduced in a dose-dependent manner.

MPS-VI patients often develop leg paresis during the course of the disease and will need neurosurgical interventions for spinal compressive myelopathy [15,16]. Accumulation of lysosomal storage in the dura mater and supporting structures (e.g. ligaments), kyphosis, scoliosis and vertebral bone deformities causing stenosis

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have been considered as aetiological explanations for spinal cord compression [15–23]. Other mucopolysaccharidoses (e.g. MPS-I, MPS-II, MPS-IV) can also induce a compressive myelopathy [24,25].

In MPS-VI cats, most likely due to the presence of the blood–brain barrier and absence of the mannose-6-phosphate receptor at this barrier [26], an insufficient amount of enzyme reaches the dura mater following long-term weekly IV ERT with 1 mg/kg rhASB, and lysosomal storage materials continue to accumulate within the dura mater [12]. Assuming that a gradual thickening of the dura mater is responsible for creating considerable compression force on the spinal cord and assuming that the dura mater will not be restored to a healthy state following weekly IV ERT with the recommended dose of rhASB, it is likely that the development of paresis will not be completely prevented with IV ERT alone in many severely affected human MPS-VI patients.

MPS-VI cats administered IV ERT from 3 months of age onwards develop circulating anti-rhASB antibodies indicating that a state of tolerance towards the foreign protein cannot be acquired with IV ERT alone past a certain stage of maturity [12]. Although not yet clearly demonstrated experimentally, it is possible that the MPS-VI cats that have seroconverted may not benefit as much from IV ERT due to interference by anti-rhASB antibodies. Immune intolerance towards systemically administered recombinant human iduronidase (rhIdu) also occurs in MPS-I dogs and for this reason a 60-day tolerisation regimen using cyclosporine (CsA), azathioprine (Aza) with a low dose of enzyme has been used with success in these dogs to prepare them for repeated IV ERT [27,28].

The significance of the immune response mounted against the recombinant enzyme used for IV ERT in human patients affected with a LSD has been recognised for some time [29]. MPS-VI patients are expected to develop antibodies towards rhASB during the course of therapy since many patients have no residual rhASB protein in their system and are unlikely to have received IV ERT from a very early age [30]. Indeed clinical studies in MPS-VI patients receiving weekly IV ERT with rhASB showed that all patients had seroconverted by 30 weeks of treatment [6,8,10]. Therefore, inducing a state of immunotolerance towards rhASB might also benefit MPS-VI patients by eliminating the possibility of anti-rhASB antibody interference with therapy and by preventing development of hypersensitivity reactions.

Since the quality of life and life expectancy of patients is expected to improve with IV ERT, adding repeated intrathecal administration of rhASB to the therapeutic regimen may eliminate the dural abnormalities and consequently lead to reduction in the incidence of cord compression and paresis. However, to minimise all risks of adverse events following intrathecal therapy or combined therapy, the immune response towards rhASB should be restrained.

In this study, we investigated whether a short-course tolerisation regimen with cyclosporine (CsA) and azathioprine (Aza) in conjunction with weekly low dose of rhASB induces immunotolerance towards rhASB in juvenile MPS-VI and unaffected cats. We subsequently examined the feasibility and safety of treating immunotolerised MPS-VI cats with weekly intrathecal injections (IT INJ) of rhASB, and verified whether this treatment was effective at reducing lysosomal storage material within the dura mater.

Materials and methods

Enzyme preparation

RhASB used for the IV ERT and for IT INJ was provided by BioMarin Pharmaceuticals Inc. Detailed preparation of the enzyme was previously described [12]. The vehicle solution used in the preparation of rhASB and for IT INJ contained 10 mM sodium phosphate, 150 mM sodium chloride and 0.025% polysorbate-80 (Tween 80). All rhASB and vehicle preparations were at pH 5.8. The concentration of the rhASB preparations was 1 and 5 mg/mL for the IV ERT and IT INJ, respectively. Before each IT INJ, one volume of rhASB (or equivalent volume of vehicle) was diluted with two volumes of Elliott's B solution (QOL Medical LLC, Seattle, WA 98021, USA).

Test animals

Cats were bred and maintained at the Institute of Medical and Veterinary Science, Adelaide, South Australia. Animal husbandry has been previously described [11,12]. All animal studies were reviewed and approved by the Women's and Children's Hospital and Institute of Medical and Veterinary Science Animal Ethics Committees.

MPS-VI diagnosis

Histological assessment of blood smears at birth and genotyping were performed as previously described [11,12,31]. The severe clinical MPS-VI phenotype (L476P homozygote) used in this study is associated with a low level of feline ASB protein [32].

Tolerisation regimen

Treatment with immunosuppressants (indicated by "T") started on day 1 of the study and ended on day 22. Starting at around 4 months of age (day 1), five MPS-VI cats (A/T; $n = 5$) and two unaffected cats (U/T; $n = 2$) received oral CsA (Table 1) every 12 h and oral Aza every third day. Each dose of CsA (Neoral Novartis, 100 mg/mL) ranged between 5 and 12 mg/kg in order to maintain a blood trough level at approximately 400 ng/mL whilst the dose of Aza was maintained at 0.3 mg/kg. The Aza preparations (10 mg/mL) were made at the Women's and Children's Hospital Pharmacy Department (Batch No. C040603, Rx No. 275581/1 and Batch No. C050430). These cats also received IV ERT with 0.1 mg/kg rhASB on days 7, 14 and 21. Four unaffected cats (U; $n = 4$) received the

Table 1
Summary of treatment with immunosuppressants and IV ERT.

Group (n =)	Phenotype	CsA + Aza doses[a] (week 1–3)	Low dose IV ERT[b] (IV ERT #1–#3) (week 1–3)	Resting period (no IV ERT) (week 4–7)	Standard dose IV ERT[c] (IV ERT #4–#24) (week 8–28)
U ($n = 4$)	Unaffected	None	✓	✓	✓
U/T ($n = 2$)	Unaffected	✓	✓	✓	✓
A/T ($n = 5$)	MPS-VI	✓	✓	✓	✓

[a] CsA (5–12 mg/kg orally) every 12 h to maintain CsA blood trough level at approximately 400 ng/mL and Aza (0.3 mg/kg orally) every third day.
[b] Weekly low dose IV ERT (0.1 mg/kg rhASB) on day 7, 14 and 21.
[c] Weekly standard dose of rhASB (1 mg/kg IV). If hypersensitivity reactions were observed during IV ERT, the infusion was stopped completely for that day and a reduced dose (0–0.25 mg/kg) was administered during the following weeks. For instance, an unaffected cat (U-1) received lower doses from IV ERT #11 to #13 (0.25 mg/kg); from IV ERT #14 to #18 (0.1 mg/kg); at IV ERT #19 (0.2 mg/kg); from IV ERT #21 to #24 (0.1 mg/kg), and PBS buffer at IV ERT #20. Another unaffected cat (U-4) received lower doses at IV ERT #10 (0.5 mg/kg) and from ERT #11 to #24 (0.1 mg/kg). An MPS-VI cat (A/T-1) received a lower dose from IV ERT #22 to #24 (0.1 mg/kg) and PBS buffer at IV ERT #21.