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Biodistribution and Pharmacodynamics of Recombinant Human Alpha-L-Iduronidase (rhIDU) in Mucopolysaccharidosis Type I-Affected Cats Following Multiple Intrathecal Administrations

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Abstract

The storage disorder mucopolysaccharidosis type I (MPS I) is caused by a deficiency in lysosomal α -L-iduronidase activity. The inability to degrade glycosaminoglycans (GAG) results in lysosomal accumulation and widespread tissue lesions. Many symptoms of MPS I are amenable to treatment with recombinant human α -L-iduronidase (rhIDU), however, peripherally administered rhIDU does not cross the blood-brain barrier and has no beneficial effects in the central nervous system (CNS). A feline model of MPS I was used to evaluate the CNS effects of rhIDU following repeated intrathecal (IT) administration. Twelve animals were randomized into four groups based on the time of euthanasia and tissue evaluation following three repeat IT administrations of 0.1 mg/kg rhIDU or placebo on Study Days 1, 4 or 5, and 9. Two days after the final IT injection, the mean tissue α -L-iduronidase (IDU) activity in the brains of the two treated animals were approximately 3-times higher (50.1 and 54.9 U/mg protein) than the activity found in normal cat brains (mean of 18.3 U/mg), and remained higher than untreated MPS1 brain at 1 month (2.4 and 4.1 U/mg protein) before returning to near-baseline levels after 2 months. This activity corresponded with decreased brain GAG concentrations after 2 days (1.4 and 2.0 mcg/mg) and 1 month (0.9 and 1.1 mcg/mg) which approached levels observed in normal animals (0.7 mcg/mg). Attenuation of GAG, gangliosides GM2 and GM3, and cholesterol reaccumulation was identified at both two days and one month following final IT injection. No adverse effects or rhIDU antibody response attributable to IT rhIDU administration were observed. IT rhIDU may be an effective means for providing enzyme replacement therapy for the central manifestations of MPS I.

Keywords

mucopolysaccharidosis; lysosomal storage disease; alpha-L-iduronidase; feline; intrathecal; enzyme replacement therapy

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1.0 Introduction

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive lysosomal storage disorder caused by deficient lysosomal α -L-iduronidase activity (IDU; E.C. 3.2.1.76) resulting in an inability to degrade the glycosaminoglycans dermatan sulfate and heparan sulfate. Unmetabolized glycosaminoglycans (GAGs) accumulate in lysosomes resulting in widespread multiorgan injury and dysfunction [1].

Recombinant human α -L-iduronidase (rhIDU) is commercially available for long-term enzyme replacement therapy (laronidase) [2], providing sustained and clinically meaningful improvements for some symptoms in patients with MPS I [3-6]. Unfortunately, peripherally administered rhIDU has no beneficial effects on the central nervous system (CNS) or skeletal manifestations of the disease such as scoliosis, kyphosis [7], cognitive deficits and spinal lesions including spinal cord compression caused by intervertebral disk compression and GAG accumulation in the meninges [8].

The intrathecal (IT) administration of rhIDU has been attempted in an effort to deliver rhIDU into the CNS. In one pre-clinical trial, 4 weekly IT injections of rhIDU resulted in a 20-300-fold increase in iduronidase activity in the brain and meninges of MPS I-affected dogs [9]. This was accompanied by a significant decrease in brain and meningeal GAG concentrations. Early IT administration of rhIDU to MPS-affected dogs every 3 months in combination with weekly intravenous rhIDU resulted in attenuation of cord compression and in significantly reduced meningeal GAG concentrations [10]. A few MPS I patients have received IT injections of rhIDU for the treatment of spinal cord compression in clinical trial settings [11-13]. Following 4 monthly IT injections, clinical improvements included reduction in baseline gait disturbances, lower limb and lumbar back pain, arm and leg tingling, ankle and wrist spasticity and mild lower leg paraparesis.

A colony of cats with naturally-occurring genetic α -L-iduronidase deficiency develop many of the same clinical characteristics observed in human MPS I patients as well as histological and biochemical evidence of lysosomal GAG storage in peripheral tissues, brain, and meninges [14]. Peripherally-administered rhIDU has previously been shown to decrease GAG storage in various organs in these animals; however, similar to humans, no beneficial effects were observed in the CNS [15]. The following study was designed to evaluate the duration of histochemical changes in the CNS of cats with MPS I following repeated IT injections of rhIDU and attempt to establish a dosing schedule for IT treatments to prevent GAG accumulation.

2.0 Material and methods

2.1 Animals

The animals used in this study were 18-27 month-old male ($N=8$) and female ($N=4$) cats affected with MPS I that were bred for these studies (University of Pennsylvania, Philadelphia, PA). These animals demonstrated similar clinical signs and biochemical derangements observed in human MPS I patients [14, 16] and have previously been shown to serve as a suitable model for enzyme replacement therapy using rhIDU [15]. The animals were provided food and water *ad libitum* and housed with 12-hour light cycles at 21° C with 12–15 air exchanges per hour. Cats were raised in the animal colony of the School of Veterinary Medicine, University of Pennsylvania under NIH and USDA guidelines for the care and use of animals in research. The experimental protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

2.2 Materials

The study drug was recombinant human α -L-iduronidase (rhIDU) provided at a concentration of 0.58 mg/mL (97 Units/mL) (Aldurazyme® [laronidase] Solution for Intravenous Infusion; BioMarin Pharmaceutical Inc.). Immediately prior to administration, the rhIDU was freshly prepared by dilution with synthetic cerebrospinal fluid (Elliott's B buffer; QOL Medical, Kirkland, WA) to achieve a final rhIDU concentration of 0.193 mg/mL. The placebo consisted of the synthetic cerebrospinal fluid (CSF) alone.

2.3 Procedure

Animals were placed into four groups in order to study the histochemical progression of disease following treatment. Group designations were assigned based on the time of euthanasia following three repeat IT administrations of rhIDU (n=2/group) or placebo (n=1/group), which were performed on Study Days 1, 4 or 5, and 9. Animals in Groups 1, 2, 3, and 4 were euthanized on Study Days 11, 37, 65, and 122, corresponding to 2, 28, 56 or 113 days after the final IT administration, respectively. On each day of enzyme administration, animals were pretreated with subcutaneous atropine 0.02 mg/kg and butorphanol tartrate 0.2-0.4 mg/kg approximately 30 minutes prior to IT injection. Anesthesia was induced with intravenous (IV) propofol 4-6 mg/kg and maintained with IV propofol throughout the procedure. The unconscious animals were placed in lateral recumbency and the area over the cisterna magna was clipped and scrubbed. A 20-gauge spinal needle was aseptically inserted into the cerebellomedullary cistern and approximately 2 mL of CSF was collected by natural flow and saved for analysis. Two animals in each group were administered rhIDU 0.1 mg/kg body weight as a slow IT bolus over 2 minutes. The remaining animal in each group was administered placebo (Elliott's B buffer). The volume of rhIDU and placebo did not exceed 3 mL. When the procedure was complete, the effect of butorphanol tartrate was reversed with IV naloxone 1.0 mg/kg.

Following each IT injection, animals were closely monitored to ensure they fully recovered from anesthesia and that each injection was well-tolerated. A veterinarian performed a physical assessment of each animal 1-2 days before and after each IT injection. Study staff recorded vital signs and food intake for 3 days after each injection. Signs of spinal cord injury such as hind limb weakness or ataxia were also recorded. Blood samples were collected for clinical chemistry and hematological evaluation on Study Days 1, 11, and immediately prior to euthanasia.

Approximately 30 minutes prior to euthanasia on Study Days 11, 37, 65 and 122, animals were treated with subcutaneous atropine 0.02 mg/kg and butorphanol tartrate 0.2-0.4 mg/kg. A catheter was placed in the cephalic vein and anesthesia was induced with 4-6 mg/kg IV propofol. Immediately prior to termination, each animal was administered 0.5 mL IV heparin (1,000 U/mL) followed by a lethal dose of IV pentobarbital (80 mg/kg). Blood, CSF and tissue were collected from each animal on the day of euthanasia for evaluation.

2.4 Blood, CSF, and Tissue Sampling

Two 1 mL blood samples were collected from all animals prior to IT injections on Study Days 1, 4 or 5, and 9. Similar blood samples were obtained from animals in Groups 1-4 prior to necropsy on days 11, 37, 65, and 122, respectively. One sample was collected into K₂EDTA-containing tubes and processed to plasma while the other sample was collected into tubes containing no preservatives and processed to serum. All samples were frozen at -60° C.

The 2 mL samples of CSF were collected immediately prior to IT administration of rhIDU and again prior to euthanasia, cooled to 2-8° C, and divided into 100 and 200 μ L aliquots

within 30 minutes after collection. Serum, plasma, and 200 μ L aliquots of CSF were shipped frozen for measurement of rhIDU concentrations and the presence of anti-rhIDU antibodies (BioMarin Pharmaceutical Inc., Novato, CA). For other analyses, CSF samples were collected into red top tubes with no preservatives. Samples were processed and analyzed within an hour after collection. The whole blood samples and 100 μ L aliquots of CSF were submitted to a clinical laboratory for biochemical and cytologic analysis, respectively (University of Pennsylvania, Philadelphia, PA).

Following euthanasia, a catheter was placed in the left cardiac ventricle and a small incision was made in the right auricle. The descending aorta was clamped with a hemostat. One liter of cold normal saline was perfused over 30 minutes. Concurrently, a craniectomy was performed to expose most of the right cerebral hemisphere and cerebellum. After the saline perfusion was complete, the right half of the brain was removed. Biopsies containing a minimum of 1 gm of tissue were obtained from the frontal, parietal, and occipital cortices and cerebellum from the right brain. Biopsy samples were immediately divided in half and placed in labeled plastic tubes on dry ice. Subsequently, each animal was perfused with approximately 1 L of cold 4% paraformaldehyde fixative for 20 minutes. The left brain was removed, placed in 400-500 mL of 4% paraformaldehyde, and stored for 36-48 hours at 2-8° C. After fixation, the left brain was sectioned and placed in cold 0.1 M phosphate buffer (pH 7.4) and the left cerebral hemisphere, cerebellum, brainstem, and proximal cervical spinal cord were shipped for histopathological processing and evaluation (Albert Einstein College of Medicine, Bronx, NY). Three left cortex samples obtained from core sections extending from the pia to white matter were cut to a thickness of 35 μ m. Blinded observers evaluated each sample for the storage of GM2 and GM3 gangliosides using immunocytochemical techniques based on peroxidase labeling methods [17, 18]. Blinded observers also evaluated each sample for unesterified cholesterol content using filipin, a highly fluorescent molecule that binds specifically to cholesterol.

Brain tissue samples from the cerebellum and frontal, parietal, and occipital cortices each weighing 100 mg were ground in 1.0 ml solution of iced 0.9% saline containing 0.2% Triton X-100, sonicated for 20 seconds to remove membranes, frozen at -80° C for 2 or more hours, and thawed in a 37° C water bath. Samples were then centrifuged to remove cellular debris and the supernatant was collected, diluted 10-fold, and tested. Protein concentration was determined by the Bio-Rad protein assay method (Bio-Rad Laboratories, Hercules, CA) using a VERSAmax Microplate Reader (Molecular Devices Inc., Sunnyvale, CA).

Brainstem, cerebellum, and cerebral cortex were also paraffin blocked, sectioned to a thickness of 5 μ m, mounted onto glass slides, stained by hematoxylin and eosin, and evaluated by a board-certified veterinary pathologist. Toluidine blue stain was used to evaluate dura mater vacuolation on 1 μ m thick plastic sections.

2.5 Analytical Methods

GAG was measured using a commercial assay (Blyscan Sulfated Glycosaminoglycan Assay Kit; Biocolor Ltd., North Ireland): 50 μ L of diluted samples were incubated with 500 μ L of Blyscan Dye Reagent on a rocker mixer for 30 min. Reactions were centrifuged at 10,000 $\times$ g for 10 min and the supernatant was removed. The pellet was resuspended in 500 μ L of Blyscan Dissociation Reagent by mixing and vortexing for 10 min. Absorbance was measured at 656 nm using a VERSAmax Microplate Reader (Molecular Devices Inc., Sunnyvale, CA) and compared against GAG standards.

Brain tissue homogenates were evaluated for α -iduronidase and β -hexosaminidase activity. To determine α -iduronidase activity,¹ 100 μ L of diluted samples were incubated with 100 μ L of 0.2 mM 4-methylumbelliferyl α -L-idopyranosiduronic acid, cyclohexylammonium

salt (4-MUI; Toronto Research Chemicals Inc., North York, Ontario, CA) in 0.1 M formate buffer (pH 3.5) at 37° C for 1-3 hours. To determine β -hexosaminidase activity, 10 μ L samples were diluted with 90 μ L dH₂O and then incubated with 100 μ L of 1.0 mM 4-methylumbelliferyl N-acetyl- β -D-glucosaminide (Glycosynth Ltd., Cheshire, England) in 0.2 M citrate-phosphate buffer (pH 4.4) at 37° C for 1 hour. Reactions were stopped by the addition of 1.8 ml of 0.32 M glycine and 0.2 M sodium carbonate (pH 10.5). Fluorescence (excitation 365 nm, emission 450 nm) was measured on a cuvette fluorometer (VersaFluor™; Bio-Rad Laboratories, Hercules, CA) and compared against a standard curve of 4-methylumbelliferone (4-MU; Sigma-Aldrich St. Louis, MO). Activity was expressed as nanomoles of 4-methylumbelliferone released/mg protein/hour.

CSF and plasma were analyzed for alpha-iduronidase activity by modifying the previously described activity assay to a microplate method. Samples and high and low positive controls were diluted 1:38 in 100 mcg/mL bovine serum albumin, 10 mM sodium phosphate, 150 mM NaCl (pH 5.6), and 25 mcL were incubated 30 minutes with 25 mcL 6 mM 4-MUI fluorogenic substrate in 0.4 M formate, 150 mM NaCl (pH 3.5). The reaction was stopped with the addition of 200 mcL 0.35 M glycine and 0.44 M sodium carbonate (pH 10) and 4-MU fluorescence was measured (366 nm excitation, 446 nm emission) on a dual-mode spectrophotometer (SpectraMax M2e; Molecular Devices, Sunnyvale, CA) and quantitated using a 4-MU standard curve from 0.10-1.25 nmol. The units of enzyme activity were calculated from the nmol 4-MU released, with one unit defined as the amount of enzyme required to cleave 1 μ mol of 4-MUI per minute. At a dilution of 1:38, this limit of quantitation was 0.005 U/mL in neat feline plasma and CSF.

CSF and serum were analyzed for the presence of anti-IDU IgG antibodies by modifying an ELISA assay previously described using dog serum [19]. Microtiter plates were coated with 100 mcL/well of 4 mcg/mL rhIDU in 100 mM sodium phosphate, 150 mM NaCl (pH 5.6) overnight at 4 °C and blocked for one hour with 300 mcL/well of Blocker Casein in phosphate buffered saline (Thermo Fisher Scientific Inc., Rockford, IL). Serial dilutions of serum and CSF were prepared in Blocker Casein and antibodies against rhIDU were captured from 100 mcL on the coated plates during incubation for 1 hour at room temperature. The IgG fraction was subsequently bound by incubating 100 mcL 1:10,000 horseradish peroxidase-conjugated goat-anti-cat IgG (Jackson ImmunoResearch, West Grove, PA) in Blocker Casein for 1 hour at room temperature and detected with a 15 min incubation of 100 mcL TMB substrate (Bio-Rad, Hercules, CA). The reaction was stopped by addition of 100 mcL 2N sulfuric acid and absorbance measured at 450 nm on a spectrophotometer (SpectraMax M2e; Molecular Devices, Sunnyvale, CA). The average absorbance was converted to OD units/mcL of serum or CSF by multiplying by the dilution of the sample and dividing by the sample well volume (100 mcL). The minimum serum dilution was 400 with a minimum limit of detection at 0.15 OD, yielding a minimum reported value of 0.6 OD/mcL. The minimum CSF dilution was 100 with a limit of detection at 0.06 OD, yielding a minimum reported value of 0.06 OD/mcL.

Comparisons for statistical difference were performed on the CSF pathology data using the Mann-Whitney test (MedCalc® Software v11.1.0) with significance set at $p < 0.05$.

¹The assay used does not distinguish endogenous α -L-iduronidase from recombinant human α -L-iduronidase.

3.0 Results

3.1 Alpha-Iduronidase Activity

Two days after the final IT injection, the mean tissue IDU activity in the brain reached 50.1 and 54.9 U/mg protein and remained at 2.4 and 4.1 U/mg protein for 1 month before returning to near-baseline concentrations after 2 months. The peak IDU activity observed after 2 days was 2.7- and 3.0-fold higher than mean IDU activity observed in normal animals (18.3 U/mg protein; $N=6$). IDU enzyme activity was not detected in the plasma or CSF of MPS I cats treated with either rhIDU or placebo at any time during the study. IDU activity from several brain regions are shown in Table 1.

3.2 Hexosaminidase Activity

Two days after the final IT injection, the mean tissue hexosaminidase activity was 3,475 and 3,522 U/mg protein which were similar to mean concentrations observed in untreated MPS I-affected animals (4,348 U/mg protein); however, after 1 month they were reduced to 2,817 and 3,085 U/mg protein which approached concentrations observed in normal animals (2,233 U/mg protein; $N=6$). Hexosaminidase activity returned to concentrations observed in MPS I-affected animals after 4 months. Hexosaminidase activity in the cerebellum and frontal, parietal, and occipital cortices are shown in Table 2.

3.3 Glycosaminoglycan (GAG) Concentrations

Two days after the final IT injection, mean tissue GAG concentrations were 1.9 and 1.4 mcg/mg protein and after 1 month they remained at 0.9 and 1.13 mcg/mg protein, approaching mean concentrations observed in normal animals (0.72 mcg/mg protein; $N=6$). GAG concentrations were 24% and 43% lower after 2 months and 4% and 33% lower after 4 months compared to GAG concentrations observed in untreated MPS I-affected animals. GAG concentrations in the cerebellum and frontal, parietal, and occipital cortices are shown in Table 3.

3.4 Immunocytochemistry and Histochemistry

Peroxidase-labeled granular storage material was observed throughout neuronal somata in all animals. In larger pyramidal neurons, storage extended into apical and larger basilar dendrites. Filipin labeling revealed the distribution of cholesterol accumulation in neuronal cell bodies was similar to the ganglioside labeling. In all rhIDU- and placebo-treated animals, the level of staining with these markers was significantly greater than normal, as GM2 and GM3 gangliosides and cholesterol accumulations are not present in neurons of normal cats at these ages [17]; however, the qualitative evaluation of GM2, GM3, and cholesterol in tissue sections under light or fluorescent microscopy by a blinded observer correctly distinguished the rhIDU treated animals vs. control animals sacrificed after 2 days and 1 month post-treatment. Compared to the placebo-treated animals, a variable but significant reduction in all three markers was observed in the rhIDU-treated animals in each group. Representative immunochemistry and histochemistry sections obtained 28 days after treatment are shown for GM2, GM3 and cholesterol (Figure 1).

3.5 Anti-IDU Antibodies in Cerebrospinal Fluid and Serum

Serum anti-IDU IgG antibodies were detected (Limit of detection, LOD = 0.6 OD/mcL) in samples collected immediately prior to euthanasia in four animals that received rhIDU infusions in Group 2 ($N=1$, 2.36 OD/mcL), Group 3 ($N=2$, 6.33 and 7.33 OD/mcL), and Group 4 ($N=1$, 0.71 OD/mcL). CSF anti-IDU IgG antibodies were detected (LOD = 0.06 OD/mcL) in samples collected immediately prior to euthanasia in three animals that received rhIDU infusions in Group 2 ($N=1$, 0.08 OD/mcL) and Group 3 ($N=2$, 0.07 and 1.9

OD/mcL). All CSF anti-IDU IgG antibody concentrations were significantly lower than the corresponding serum antibody levels from the same animals. Comparisons of actual antibody levels between animals were not statistically valid due to the limited number of samples, however, the trends suggest that low levels of antibodies are produced by 30 to 60 days after the final enzyme administration.

3.6 Cerebrospinal Fluid Pathology

CSF analysis prior to IT injection showed pre-existing abnormalities in protein concentrations and cellular differentials. The majority of MPS I cats (83%) had albuminocytologic dissociation characterized by increased protein concentration in the absence of cellular pleocytosis: eight (66%) had mild to moderate increases in protein concentration (35- 46 mg/dL), two (17%) had protein concentrations that were marginally increased (31 mg/dL), and the remaining two (17%) had protein concentrations below the reference limit (≤ 30 mg/dL). In contrast to protein concentrations, nucleated cell counts were within the reference interval for eleven (92%) MPS I animals. Four animals (30%) had an increased percentage of neutrophils however which were attributed to mild inflammation associated with neurodegenerative disease.

Following IT injections, protein concentrations did not differ significantly from pre-treatment concentrations ($p=0.72$). No significant differences in protein concentrations were detected between the rhIDU treatment or control groups before and after injections ($p=0.50$ and $p=0.78$, respectively). In post-injection groups, including the control group, very mild pleocytosis (6/mcL) was present in CSF from four animals (30%) although no significant difference between nucleated cell counts in pre- and post-injection groups was detected ($p=0.06$). Nucleated cell counts did not significantly differ before and after rhIDU or placebo injections ($p=0.10$ and $p=0.53$, respectively). The cellular differential in nine cats (75%) showed abnormal percentages of neutrophils and/or eosinophils compared with only four cats (30%) before injections. While no significant differences were detected in cell counts or protein concentrations before and after control and therapeutic injections, an abnormal cell differential was present in 75% of MPS I cats after IT injections, including control injections.

3.7 Safety

No significant changes in behavior or vital signs were observed during the study. Body weights generally remained stable or decreased slightly during the treatment phase of the study. After day 9, body weights remained stable or increased slightly. No adverse events that appeared to be related to rhIDU treatment were identified.

Gait abnormalities were observed in rhIDU-treated ($N=6$) and placebo-treated animals ($N=2$). These abnormalities were largely characteristic of cats affected with MPS I except for apparent front limb weakness in a two rhIDU-treated and one placebo-treated animal. Limb weakness worsened in all three animals after each treatment. One rhIDU-treated animal remained symptomatic after 1 month while two were asymptomatic after 2 months.

Histological evaluation of the brainstems at each time point confirmed that all animals showed evidence of a storage disorder, characterized by the accumulation of pale material primarily within the neuronal cytoplasm but also within the cytoplasm of glial cells and meningeal fibroblasts with highly vacuolated macrophages/microglia aggregating around blood vessels. Additional brainstem findings included minimal white matter vacuolation, axonal dilation, and axonophagia in some but not all animals.

White matter changes in the brainstem consisted of minimal-to-moderate vacuolation, minimal-to-mildly swollen axons with dilated myelin sheaths, and rare axonophagia such as

digestion chambers, which is consistent with Wallerian degeneration (Table 4). These findings were uncommon on day 11 but became progressively more frequent and severe on days 37, 65, and 122 and were present in all placebo-treated cats but only in 2 rhIDU-treated cats on day 65 and 1 rhIDU-treated cats on 122. As the incidence and severity of the white matter lesions were similar between the vehicle- and rhIDU-treated cats regardless of time point, they were not interpreted as rhIDU treatment-related.

No clinically-significant changes in hematology or clinical chemistry parameters were observed.

4.0 Discussion

4.1 Efficacy of Intrathecal rhIDU

The treatment of MPS I-affected cats with repeated IT administered rhIDU demonstrated that the amount of enzyme activity entering the brain exceeded levels observed in normal animals. These elevated enzyme levels were associated with a maximum reduction in neuronal storage within 28 days with declining effects after 56 and 112 days. These data are consistent with previous observations [9, 20] and further support IT enzyme replacement for treating the CNS manifestations of MPS I. The IDU activity in the cerebral cortex of treated MPS1 cats 48 hours following IT administration was approximately 50% of that found in normal dog brain following intrathecal administration of 1 mg and may represent differences between CSF volume and drug distribution between species as well as differences in methodology [9]. In our study, we did not examine the distribution of rhIDU into deeper brain structures.

The lysosomal (or tissue) half-life of rhIDU cannot be precisely determined, but appears to be approximately 6-7 days because a 3-fold higher than normal enzyme level (300%) elevation was reduced to about 20% over a period of 28 days. This is consistent with the 7-day half-life estimated by confocal immunofluorescence from IT studies in normal rats (unpublished data on file). Due to its rapid clearance, rhIDU was not observed in either the plasma or CSF, as expected, even at the earliest timepoint of 2 days (Study Day 11) following the final IT enzyme administration (Study Day 9). GAG concentrations in the brain began to increase after 56 days although they remained below baseline levels of untreated MPS I-affected cats.

4.2 Pharmacodynamics of Intrathecal rhIDU

Increased IDU was measured in several regions of the brain and was associated with increased clearance of the primary (GAG) and secondary storage products. One month after the final IT dose of rhIDU, enzyme activity remained higher than baseline levels and was sufficient to attenuate GAG, GM2, GM3, and cholesterol reaccumulation. Concentrations of GAG in sampled brain tissue were within normal levels at 2 and 28 days after the last IT injection of rhIDU and remained below baseline levels for up to 111 days after the last IT injection. GAG reaccumulation occurred but did not reach baseline levels during the 4-month period following the last IT dose. The secondary storage products GM2, GM3, and cholesterol were reduced during the 1-month period following the last dose and returned to baseline by 2 months.

4.3 Safety of Intrathecal rhIDU

The dose level of 0.1 mg/kg body weight was chosen as this has been used safely in intrathecal administration to MPS I dogs with demonstrated pharmacodynamic activity [9,10]. In published reports, a total dose of 1 mg was administered to beagle/Plott hound mixed breed dogs which weigh approximately 5-10 times more than cats. The dose regimen

of three doses over a 9-day period was chosen to maximize the amount of drug exposure prior to the development of a potential antibody mediated immune response as a result of the administration of a heterologous protein (rhIDU).

No adverse effects were attributed to IT rhIDU administration or any specific antibody response toward rhIDU. Based on the dose regimen used in this experiment (ie three repeat IT administrations of enzyme within 9 days) this was an expected outcome and is evidenced by the low levels of serum and CSF rhIDU-specific antibodies, which was observed between 30 to 60 days post final IT administration. No significant changes in clinical hematology or chemistry parameters were observed. Elevated protein concentrations in the CSF were observed in all animals irrespective of treatment and appeared to be consistent with MPS I. Interruption of normal CSF flow and absorption can also cause albuminocytologic dissociation and should be considered as a primary or contributing cause given that ventriculomegaly (hydrocephalus) is frequently encountered in MPS I and VI patients [21, 22]. While it is possible that the increase in abnormal differential counts may be attributable to the IT injections, it is also possible that it merely reflects MPS I disease progression or mild acute inflammation which could be a consequence of performing IT injections and/or an index of neurodegenerative disease progression. The incidence and severity of observed lesions was similar between treatment and placebo groups and appeared to be unrelated to rhIDU and may have resulted from flexion of the neck at the time of CSF collection or during IT injections, local trauma associated with the IT injections, or could be an intrinsic lesion of cats with MPS I.

IT treatment with rhIDU resulted in the appearance of serum anti-IDU IgG antibodies between study days 37 and 65 but was reduced by study day 122. Significantly lower concentration of anti-human IDU IgG antibodies were detected in CSF. In several animals, anti-human IDU IgG antibodies detected in CSF may have been caused by contamination with blood. Among animals with anti-human IDU antibodies, there was no association with histopathological observations in the CNS. Abnormal gait and posture noted in three animals occurred several days after IT injection, suggesting these observations were not due to acute spinal cord trauma; however, persistent front limb weakness observed one animal after 1 month may have been at least partly attributable to the IT procedure.

5.0 Conclusions

The IT administration of rhIDU to MPS I-affected cats was generally well-tolerated. GAG storage in rhIDU treated MPS I cats was reduced to near normal levels and did not reaccumulate within 1 month after the final IT injection. Although GAG concentrations increased 2-4 months after the final IT injection, they remained lower than those of placebo-treated animals.

These data suggest that IT administration of rhIDU, with potential dosing every 2-3 months, may provide benefit for the treatment of CNS disease in MPS I patients, although the efficacy of administration at the lumbar region remains to be established..

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Figure 1. Brain GM2, GM3 immunochemistry and cholesterol histochemistry. The secondary storage of GM2 (A and B) and GM3 (C and D) gangliosides, and of cholesterol (E and F) were reduced at 28 days following the final intrathecal injection of recombinant human alpha-L-iduronidase. Untreated MPS1 animal are represented by A, C, and E while intrathecally treated animals are B, D, and F.

Table 1

IDU Activity (U/mg protein) in Tissues from Several Brain Regions.

Group	Animal	Treatment	Brain Region			Analyses Day	Mean (SD)
			Frontal	Parietal	Occipital	Cerebellum	
1	1	rhIDU	41.48	55.32	58.90	44.74	50.1 (8.32)
	2	rhIDU	60.00	45.62	47.88	66.00	54.9 (9.74)
	3	placebo	2.66	0.33	0.30	0.44	0.93 (1.15)
2	1	rhIDU	2.77	2.34	2.08	2.37	2.39 (0.28)
	2	rhIDU	4.34	4.07	3.94	3.95	4.07 (0.19)
	3	placebo	0.16	0.15	0.16	0.15	0.16 (0.00)
3	1	rhIDU	0.55	0.48	0.47	0.47	0.49 (0.04)
	2	rhIDU	0.90	1.01	0.85	0.78	0.88 (0.10)
	3	placebo	0.34	0.32	0.37	0.37	0.35 (0.02)
4	1	rhIDU	0.18	0.13	0.16	0.17	0.16 (0.02)
	2	rhIDU	0.17	0.15	0.19	0.15	0.17 (0.02)
	3	placebo	0.15	0.21	0.18	0.16	0.17 (0.03)

Historical normal (N=6) and affected control concentrations (N=6) were 18.3 (2.29) and 0.35 (0.25), respectively.

Table 2
Hexosaminidase Concentrations (U/mg protein) in Tissues from Several Brain Regions.

Group	Animal	Treatment	Brain Region			Analysis Day	Mean (SD)
			Frontal	Parietal	Occipital	Cerebellum	
1	1	rhIDU	3168	3640	4129	2963	3475 (520)
	2	rhIDU	4091	3695	2807	3496	3522 (537)
	3	placebo	3975	3238	3556	3467	3559 (308)
2	1	rhIDU	2931	2768	2790	2781	2817 (76)
	2	rhIDU	3282	3276	2953	2830	3085 (229)
	3	placebo	4454	4072	5094	4399	4505 (427)
3	1	rhIDU	3962	3487	3717	3370	3634 (262)
	2	rhIDU	3896	3965	3743	3656	3815 (141)
	3	placebo	4692	5915	6225	6197	5757 (724)
4	1	rhIDU	3867	4244	4302	3783	4049 (262)
	2	rhIDU	3627	3971	3919	4136	3913 (212)
	3	placebo	5008	5236	5008	5104	5089 (108)

Historical normal (N=6) and affected control concentrations (N=6) were 2,233 (229) and 4548 (750), respectively.

Table 3

GAG Concentrations (µg/mg protein) in Tissues from Several Brain Regions.

Group	Animal	Treatment	Brain Region			Analysis Day	Mean (SD)
			Frontal	Parietal	Occipital	Cerebellum	
1	1	rhIDU	1.59	2.11	2.67	1.35	1.93 (0.59)
	2	rhIDU	2.03	0.86	1.41	1.13	1.36 (0.50)
	3	placebo	19.91	21.66	22.67	20.06	21.1 (1.32)
2	1	rhIDU	0.22	0.25	0.28	2.87	0.90 (1.31)
	2	rhIDU	1.46	1.45	1.14	0.49	1.13 (0.45)
	3	placebo	30.87	30.85	46.23	34.82	35.7 (7.27)
3	1	rhIDU	17.97	16.80	24.79	19.15	19.1 (3.34)
	2	rhIDU	13.55	14.60	12.60	16.13	14.3 (2.05)
	3	placebo	30.77	24.30	31.94	25.19	27.3 (3.78)
4	1	rhIDU	13.51	14.13	20.29	19.54	16.9 (3.54)
	2	rhIDU	20.97	24.50	26.97	24.59	24.2 (2.48)
	3	placebo	35.06	32.63	36.42	33.74	34.5 (1.64)

Historical normal (N=6) and affected control concentrations (N=6) were 0.72 (0.35) and 25.3 (5.28), respectively.

Table 4

Evidence of Wallerian Degeneration in Brainstems of Cats with MPS I.

Animal	Group 1 (Day 11)			Group 2 (Day 37)			Group 3 (Day 65)			Group 4 (Day 122)		
	1	2	3	1	2	3	1	2	3	1	2	3
Brainstem Findings*												
White matter vacuolation												
<i>Minimal</i>	+	-	-	-	-	-	+	-	-	-	+	-
<i>Mild</i>	-	-	-	-	-	-	-	-	-	-	-	+
<i>Moderate</i>	-	-	-	-	-	+	-	+	+	-	-	-
Axonophagia												
<i>Minimal</i>	-	-	-	-	-	+	+	-	+	-	+	+
<i>Mild</i>	-	-	-	-	-	-	-	+	-	-	-	-
<i>Moderate</i>	-	-	-	-	-	-	-	-	-	-	-	-
Swollen axons												
<i>Minimal</i>	-	-	-	-	-	-	-	-	+	-	-	-
<i>Mild</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Moderate</i>	-	-	-	-	-	-	-	-	-	-	-	-

* Greatest lesion severity score in 3 slides examined.