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Replacing the Enzyme α -L-Iduronidase at Birth Ameliorates Symptoms in the Brain and Periphery of Dogs with Mucopolysaccharidosis Type I

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Abstract

Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disease caused by loss of activity of α -L-iduronidase and attendant accumulation of the glycosaminoglycans dermatan and heparan sulfates. Current treatments are suboptimal and leave residual disease including corneal clouding, skeletal deformities, valvular heart disease and cognitive impairment. We treated neonatal mucopolysaccharidosis I dogs with intravenous recombinant α -L-iduronidase replacement therapy at the conventional 0.58 mg/kg or a higher 1.57 mg/kg weekly dose for 65–81 weeks. In contrast to previous results in animals and patients treated at a later age, all animals failed to mount an antibody response to enzyme therapy, consistent with neonatal tolerization. The higher dose of enzyme led to complete normalization of lysosomal storage in liver, spleen, lung, kidney, synovium and myocardium, as well as in the hard-to-treat mitral valve. Cardiac biochemistry and

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Author contributions: NME and PID conceived and designed the study, analyzed data and wrote the manuscript. ADD performed experiments and data analysis and wrote the manuscript. MFM, CAV, and AF-W performed pathology. CHV, WG, and EAR performed radiology and MRIs. MP, SS, AHC and SL performed biochemistry. JKJ and EMS conducted animal work. KKL, JDP, and JAW conducted veterinary neurology procedures and support. WAW and LEM conducted veterinary cardiology procedures and support. RDW, DMB and AMB conducted veterinary ophthalmology procedures and support.

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function were restored, and there were improvements in clinical and radiographic signs of skeletal disease. Glycosaminoglycan levels in the brain were normalized after intravenous enzyme therapy, with or without intrathecal treatment with recombinant α -L-iduronidase. Histopathologic evidence of glycosaminoglycan storage in the brain was ameliorated with the higher dose intravenous therapy and further improved by combined intravenous and intrathecal therapy. These findings argue that neonatal testing and early treatment of patients with mucopolysaccharidosis I may more effectively treat the disease.

Summary—This work documents from birth ERT therapy in a large animal model of MPS I, and details substantial clinical response in this model in what were previously intractable and difficult to treat tissues, all of which argues for neonatal testing and evaluation of neonatal initiated therapy in MPS I.

Keywords

Mucopolysaccharidosis I; lysosomal storage diseases; iduronidase; enzyme replacement therapy; tolerance; Hurler syndrome; Scheie syndrome; disease models; animal

Mucopolysaccharidosis type I (MPS I) (OMIM 607014-16) is a lysosomal storage disease characterized by organomegaly, corneal clouding, skeletal deformities, cardiovascular disease, respiratory inadequacies, and varying degrees of central nervous system involvement. Phenotypes range from severe (Hurler syndrome) to attenuated (Scheie syndrome), and depend on the degree of residual α -L-iduronidase (iduronidase, IDU, EC 3.2.1.76) (1). Left untreated, severely affected individuals often succumb to disease in the first decade while attenuated individuals may live well into adulthood (2). Hematopoietic stem cell transplantation is used for the severe (Hurler) form of MPS I, and works by providing a source of naturally-secreted enzyme. Transplanted cells of the macrophage lineage distribute to brain by passage across the blood-brain barrier (3). Hematopoietic stem cell transplantation can impede the progression of intellectual decline if performed early in the disease course (4). Age at treatment varies, but for the severe form of MPS I (Hurler disease), the median age at diagnosis is 9.6 months, 3 months on average after the onset of symptoms (5,6). However, even with transplantation, dysfunction persists in communication, motor skills, socialization and activities of daily living (7).

Recombinant human α -L-iduronidase (rhIDU) is used as enzyme replacement therapy (ERT) primarily for the attenuated (Hurler-Scheie and Scheie) forms of the disease. Current practice requires that ERT be administered intravenously (IV) at 0.58 mg/kg weekly, based on studies in the canine model of MPS I (8). The canine MPS I model is a naturally-occurring large animal model which does not produce IDU, stores GAG, and displays an MPS I phenotype including coarse features, umbilical hernia, corneal clouding, cardiac muscle hypertrophy and valvular thickening, and spinal cord compression (9,10). Administration of ~0.5 mg/kg rhIDU to MPS I dogs resulted in biochemical and clinical improvement in systemic manifestations of disease, and led to the 0.58 mg/kg dose selected for human ERT trials (8). The approved regimen of 0.58 mg/kg weekly ERT improves joint mobility and reduces urinary GAG levels and liver size (urinary GAG excretion and liver size are clinically useful markers of overall GAG storage), among other benefits. However, clinical studies have documented that it does not completely correct cardiac or skeletal abnormalities and is not expected to prevent cognitive deterioration, since previous ERT studies in the dog showed little discernable enzyme activity in the brain (8). Over the long-term, individuals with attenuated forms of MPS I maintain clinical improvements in organomegaly, joint mobility, and pulmonary function, but develop progressive corneal clouding, cardiac valvular disease, and spinal cord compression (11). The extent to which individuals with attenuated MPS I develop cognitive impairments is not known, and a

natural history study to measure intellectual function over time in these patients is ongoing. In the canine model, even a larger 2 mg/kg weekly dose of ERT was not sufficient to clear accumulated GAG from the heart valve in older animals, though it was able to improve histologic evidence of lysosomal storage efficacy(12,13). It is possible that some MPS I pathology, including valvular disease, may be difficult or impossible to reverse.

While some MPS I disease is not readily reversed by IV ERT, prevention may be easier to achieve. In particular, we were intrigued by reports that early or high-dose IV ERT could treat lysosomal storage in the brain in mice (14–20). We therefore treated MPS I dogs shortly after birth to determine whether hard-to-treat disease including cardiac valvular disease, skeletal disease, and corneal clouding, would respond to early initiation of therapy, and whether treating them would require a higher dose. We also sought to compare early, high-dose IV ERT to ERT administered directly into spinal fluid (intrathecally, IT) (21,22) for treatment of lysosomal storage in the brain.

RESULTS

MPS I dogs treated from birth are tolerant to enzyme replacement therapy

Dogs affected with MPS I were diagnosed at birth by PCR and enzyme activity assay and received ERT with rhIDU initiated between 3 to 23 days of age (Fig. 1). Twelve untreated MPS I dogs (referred to as “affected”) and twelve normal dogs were also included for comparison to treated dogs. Baseline titers of serum antibody to rhIDU were measured by ELISA in samples taken before the first treatment and ranged from 0.031 to 0.647 OD units (405 nm) per μL serum. Final titers taken at necropsy ranged from 0.249 to 0.608 OD units (Fig. 1C). These results suggest that all animals developed immunological tolerance to rhIDU. Previous studies established a cut-off for clinical tolerance of < 20 OD units/ μL (12,13).

Enzyme replacement therapy from birth yields high enzyme activity and normalized GAG storage

Tissues were evaluated 48 hours after the last dose of IV rhIDU. IDU activity was increased in treated animals in a dose-dependent fashion (Table 1). Tissue GAG in liver, spleen, lung, myocardium, renal cortex, and renal medulla were at or below normal range of values in all dogs treated from birth, including both the higher and lower-dose groups (Fig. 2A–F). There was no significant difference between dosage groups in any of the tissues ($p \leq 0.60$). Liver size, as percent of body weight, was lower in the treated group than in MPS I untreated dogs ($p < 0.0001$) and was fully normalized ($p \leq 0.54$) in both dosage groups ($6.50 \pm 0.81\%$ in untreated MPS I dogs, $n = 5$; $3.10 \pm 0.35\%$ in 0.58 mg/kg treated dogs, $n = 8$; $3.52 \pm 0.71\%$ in 1.57 mg/kg treated dogs, $n = 4$; and $3.53 \pm 0.46\%$ in normal dogs, $n = 5$).

MPS I dogs treated from birth have normal heart valves

With IV rhIDU treatment beginning at birth, cardiac valvular GAG concentrations were significantly reduced ($p < 0.0001$) to normal values, with a stronger response in the higher-dose treated animals (Fig. 3A). Mitral valve GAG levels in treated dogs in this study were lower than those seen in a previous study in which rhIDU-tolerant adult animals treated with 2.0 mg/kg weekly IV rhIDU averaged 68.9 ± 31.3 μg GAG per mg dry tissue ($n = 3$) (13).

By echocardiography, the average thickness of the anterior mitral leaflet in all treated animals was similar to that of normal animals ($p \leq 0.90$) and significantly less ($p \geq 0.0009$) than that of untreated animals (Fig. 3B–E). Additional parameters evaluated included ejection fraction, left ventricular thickness, and aortic diameter (table S1). Histopathology of the tricuspid valve also showed decreased lysosomal storage in treated animals (Fig. 3F–I).

The valve in the high-dose treated animals was indistinguishable from those in normal animals, while trace GAG storage was evident in the low-dose treated dogs.

MPS I dogs treated from birth have more normal skeletons and joints

We took radiographs at ages 12.5 to 21.5 months (Fig. 4), and performed clinical assessments, including photographs and video, before euthanasia. In agreement with previous studies (23), we found abnormalities of the spine in untreated dogs, including fusions of vertebral bodies in cervical, thoracic, and lumbar spine segments. In contrast to previous studies, our MPS I affected animals did not show significant signs of appendicular effusions or dysplasia. This discrepancy is unexplained but may be related to different background genetics between different canine MPS I colonies.

By radiography, treated animals had reduced skeletal disease with reduced physal abnormalities and normal intervertebral spaces (Fig. 4O).. Animals treated from birth with 1.57 mg/kg IV rhIDU displayed no intervertebral disc space narrowing. In dogs receiving 0.58 mg/kg weekly IV rhIDU (with or without IT rhIDU) intervertebral disc space narrowing was seen commonly (7 of 8 dogs). Radiolucencies, consistent with persistent physal or apophysal endochondral remnants (23) appeared commonly at the tibial tuberosity or humeral tubercle or head and less frequently at vertebral body endplates. These were a consistent finding in our untreated MPS I dogs, in all eight 0.58 mg/kg treated dogs and in one 1.57 mg/kg treated dog.

IDU was detected at therapeutic activity in rib cartilage in both dosing groups (Table 1). Histopathology showed that treated animals had decreased lysosomal storage in both rib cartilage (Fig. 5A–C) and in the patellar synovium (Fig. 5D–F) compared to untreated MPS I dogs. All untreated dogs displayed extreme joint laxity, most notable in the carpus (Fig. 5L), splayed toes (Fig. 4L), a stiff gait, an arched back, a characteristically upturned nose (Fig. 5I), and limited voluntary cervical range of motion. Three of the 1.57 mg/kg treated dogs (I180, I196, and I200) had no signs of clinical signs of joint laxity, splayed toes, stiff gait, arched back, or upturned nose. All the treated animals had normal appearing craniums with a normal mild stop as opposed to the pronounced stop (the canine equivalent of a depressed nasal bridge) and shortened and upturned muzzle seen in affected animals (Fig. 5G–I). Additionally, the treated dogs lacked the severe overextended carpal, metacarpal, tarsal and metatarsal joints of affected dogs (24) (Fig. 4C, F, I, and L, and 5J–L).

On blinded ophthalmologic examination, corneal clouding was rated as severe in all four untreated MPS I dogs. Corneal clouding in dogs receiving 0.58 mg/kg weekly IV ERT was rated as severe in four dogs, moderate in two dogs, and mild in two dogs. In dogs receiving 1.57 mg/kg weekly IV ERT, corneal clouding was scored as moderate in one dog and mild in two dogs (one dog was unscored). Histopathology analysis of corneal tissue exhibited some improvement in lysosomal storage in dogs receiving 1.57 mg/kg, but in dogs treated at the conventional 0.58 mg/kg dose, lysosomal storage was similar to the untreated group (Fig. 5M–O). Anterior chamber flare, indicating turbidity of the aqueous humor caused by increased protein levels and/or cells, was seen in all untreated individuals, but was seen in only one of 12 treated animals.

Brain GAG storage is reduced with IV ERT from birth

All IV and IV/IT treatment groups showed normal mean brain GAG at end-study (Fig. 6). IDU activity in dogs that received IV/IT rhIDU was above normal throughout the brain (mean 90.8 ± 24.2 units/mg protein) (Fig. 6), while activity in IV ERT-treated brain ranged from 0.10 to 0.25 units/mg. Normal IDU activity was 8.26 ± 0.75 . Analysis of functional regions of the brain (frontal cortex, cerebellum, basal ganglia and thalamus, hippocampal

formation, and brain stem) showed above-normal iduronidase levels in IV/IT subjects in each region at 48 hours following the final IT rhIDU dose (fig. S1). This contrasts with subnormal but evenly distributed iduronidase activity among brain regions in dogs receiving IV ERT alone. GAG was reduced in each brain region compared to untreated MPS I animals in dogs treated with IV/IT or 1.57 mg/kg IV rhIDU, reaching statistical significance (by *post-hoc* Dunnett's test) in all areas except the basal ganglia (with thalamus) and brain stem in IV/IT-treated animals (table S2). Toluidine blue staining of the parietal cortex showed no GAG storage in perivascular or glial cells of IV/IT-treated MPS I dogs and reduced GAG storage in 1.57 mg/kg IV rhIDU-treated dogs (Fig. 6). Neuronal storage, which was principally lipid, was reduced with IV/IT rhIDU, but IV rhIDU alone had no effect (Fig. 6E). Hexosaminidase and β -glucuronidase are secondarily elevated in MPS I animals. Biochemical assays for these enzymes showed substantial elevations in activity in frontal cortex of untreated MPS I dogs, which was significantly reduced with IV/IT rhIDU (fig. S2).

Magnetic resonance imaging of the brain showed few abnormalities in MPS I dogs when they were compared to normal animals at 12–18 months. Ventricular size, measured by frontal-occipital horn ratio (FOR), was enlarged in two untreated MPS I (FOR 0.46 ± 0.064) as compared to two age-matched normal animals (0.30 ± 0.014). IV rhIDU-treated dogs (both doses) had mean FOR of 0.38 ± 0.056 (n=5); IV/IT rhIDU-treated dogs 0.39 ± 0.028 (n=2). Cortical atrophy was rated as mild or moderate in two untreated MPS I dogs, moderate or absent in five IV rhIDU-treated dogs, mild in two IV/IT rhIDU-treated dogs, and absent in two normal dogs. Three untreated MPS I dog showed bilateral and one untreated MPS I dog showed unilateral 3–5 mm focal contrast enhancing regions lateral to the mid mesencephalon tegmentum (Fig. 6A). Histopathology of the masses revealed fibrous meningiomas, with infiltration by foamy interstitial cells (Fig. 6B and 6C). These were not seen in treated or normal animals. Meningiomas have been reported in a feline model of MPS I, but their significance is unknown (25).

MPS I dogs tolerate ERT when initiated just after birth

In all, our dogs received 831 IV rhIDU infusions without serious adverse reactions. The first two pups were treated at 7–8 hours after birth, and died 2 and 6 days after the initial treatment. No obvious cause of death was found on gross post-mortem examination. Subsequent IV rhIDU treatments were begun between days 3 to 23 days after birth. Mild reactions to the infusions included restlessness and diarrhea. We noted no fevers or changes in blood counts or serum biochemistries. There was a small amount of pale-staining lipid (lacking the character of GAG) in the cytoplasm of cortical brain perivascular cells in three IV/IT rhIDU-treated dogs, one 0.58 mg/kg and one 1.57 mg/kg IV rhIDU-treated MPS I dog. A 1.57 mg/kg IV-treated and an untreated MPS I dog developed clinically stable mild to severe cerebellar ataxia, possibly due to an earlier infection with canine distemper virus. Infection could not be confirmed in these dogs due to the chronicity of signs; however, infection was confirmed in other dogs in the colony during this period.

DISCUSSION

Our findings suggest that the combination of early and higher-dose treatment can increase effectiveness of ERT for MPS I. The only non-CNS pathology evaluated that did not show complete or significant resolution or improvement was corneal disease. Although there was histological improvement in the corneas of high dosed dogs, clinical corneal clouding was not prevented, possibly reflecting its relative inaccessibility. Uveitis was prevented in treated versus untreated dogs, but further study of ocular disease in MPS I canines is required to more fully appreciate the importance of these findings.

Higher than conventional doses of ERT have been tested in MPS I patients (26). However, no subjects were treated beginning from birth, and 97 to 100% of the children developed antibodies to ERT, both of which may have limited the efficacy of the higher dose. In our previous study of a 2.0 mg/kg weekly dose in MPS I dogs, improvement in the histopathology of the heart valve was observed only when immune tolerance to rhIDU was present (13). Even in tolerant dogs, however, GAG levels in the heart valve did not decrease. In the current study, the improvement in GAG storage and thickness of the valve in MPS I dogs treated with 1.57 mg/kg weekly from birth suggests that GAG deposition can be prevented. The absence of an immune response to rhIDU in these dogs may have also played a role in the greater effectiveness of the ERT.

Our study did not address how soon after birth therapy must begin in order to have the greatest effect. Treatment was initiated at day 23 in one animal (I-141), which had the worst score/evaluation in a number of criteria, including mitral valve thickness, corneal clouding, carpal laxity, mitral GAG, and disc disease. This finding suggests that delaying therapy by 2–3 weeks, when puppy weight increases more than 2.5-times, may adversely affect canine disease, although further study is required to address this.

Early treatment with IV with or without IT rhIDU led to quantitative reduction or normalization of GAG storage in the brain. Histologically, however, the treatment with both IT and IV rhIDU was more effective than IV rhIDU alone, and led to prevention of cortical GAG accumulation and reduction of neuronal lipid. Previous work in neonatal mouse models of several lysosomal storage diseases has shown improvement of brain disease following IV ERT (14–17,27). In newborn mice, the blood-brain barrier contains mannose 6-phosphate receptors, which decline with increasing age (28), perhaps enabling enhanced trafficking of enzyme across the blood-brain barrier at a younger age. Whether this mechanism is at play in the canine neonate is unknown at this time.

Early treatment of lysosomal storage diseases has been shown to improve outcomes, including some cardiac manifestations, in animal models of MPS I and other closely related MPS disorders and human MPS I patients (18–20,23,29–39). The majority of MPS I patients who are under 5 years old at the start of IV ERT and who showed left ventricular hypertrophy at baseline show normalized left ventricular mass at 1 year (26). In MPS I mice and dogs receiving gene therapy at birth, high IDU serum levels (at least ~500 U/ml) prevented development of cardiac disease (34,40). Hematopoietic stem cell transplantation likewise can ameliorate but does not prevent cardiac, skeletal or corneal disease in large animal models; in these studies, animals received transplants after the neonatal period (41–44).

The improved outcomes resulting from early intervention in lysosomal storage diseases increase the urgency for the implementation of newborn screening for these disorders. A newborn screening method using dried blood spots can reliably distinguish MPS I affected individuals, carriers and controls (45–47). A pilot study of newborn screening for Pompe disease in Taiwan resulted in patients diagnosed between 9 and 22 days of age, suggesting that early initiation of therapy for lysosomal storage disease patients identified via newborn screening is feasible (48).

MATERIALS AND METHODS

Dogs were maintained at Iowa State University (ISU) according to IACUC protocols, and NIH and USDA requirements. The colony was founded by MPS I Plott hounds, beagles, and cross-bred dogs (49). Breeding stock originated from Harbor-UCLA, with additional breeding animals from Kathy P. Ponder and Mark E. Haskins. Diagnosis was by plasma

iduronidase assay and PCR (9,13). Dogs began rhIDU at 3 to 23 days of age. Two died within 7 days of birth, leaving 12 dogs completing the study: 4 dogs received 0.58 mg/kg weekly IV rhIDU plus quarterly 0.058 mg/kg (maximum 1 mg) IT injections, 4 dogs received 0.58 mg/kg rhIDU weekly IV injections, and 4 dogs received 1.57 mg/kg rhIDU weekly IV injections. Controls included untreated MPS I affected and carrier dogs, including 3 untreated MPS I and 5 normal dogs previously published (13). Affected controls were age-matched unless no significant age-dependent differences were observed. Normal animals ($n = 12$) were 8–78 (23.9 ± 18.9) months and untreated affected animals ($n = 12$) were 13–28 (19.0 ± 6.52) months.

Administration of enzyme replacement therapy

Lots 295292, 4986252, 5930176, and P10502 of rhIDU in buffer (100 mM sodium phosphate, 150 mM sodium chloride, 0.001% polysorbate 80, pH 5.5 – 5.6, donated by BioMarin Pharmaceutical) were stored at 4°C. Enzyme was prepared within an hour of infusion by dilution in 0.9% saline to 10 mL/kg weight (13). Conventional dosage (0.58 mg/kg) dogs received 2.5% of the dose during the first hour, and 97.5% of the enzyme over the second and third hours. For 1.57 mg/kg dosage, 2.5% dose was administered during the first hour, 12.5% in the second hour, and 85% in the third hour. No immunosuppressive was used. Dogs received 2.2 mg/kg diphenhydramine before each treatment.

For IT injections rhIDU was diluted in Elliotts B synthetic CSF (Ben Venue Laboratories) within an hour of administration (13). rhIDU 0.058 mg/kg was administered over 5 minutes directly into the cerebellomedullary cistern in anesthetized dogs as published (21,22). CSF was collected prior to IT injections, and blood was collected before each IV and IT treatment for anti-iduronidase IgG antibody ELISA and routine CSF analysis.

Biochemical measurements

Euthanasia was by IV barbiturate overdose 48 hours after the last rhIDU IV infusion. Tissues were harvested immediately, frozen and shipped on dry ice to LA BioMed at Harbor-UCLA. Tissue homogenates were evaluated with 4-methylumbelliferyl- α -L-iduronide substrate (Glycosynth) as described (8,13), with incubation at 37°C for 1 hour. Units of IDU activity (nmoles 4MU released/h) were normalized to mg protein. The Björnssen dye binding method (50) was used with modifications (8,13) for GAG measurements (μ g GAG/mg dry weight).

Histopathology

Immediately after euthanasia, systemic tissues (other than brain) were fixed in 10% (vol/vol) neutral buffered formalin, then paraffin embedded, sectioned, and stained with hematoxylin and eosin. Sections (4 μ m) were scored blindly from 0–4 as previously published (13). Brain tissue sections (parietal cortex) were fixed in 4% paraformaldehyde/2% glutaraldehyde in neutral phosphate buffer. Thin sections were stained with toluidine blue for light microscopy. Neurons were identified by morphology.

Clinical Studies

Mobility and posture were documented at the end of the study via video and photography. Posture, gait, joint mobility and neurological findings were assessed. Standard radiographs (lateral and ventrodorsal (*i.e.* cranialcaudal) views of skull, cervical spine, pelvis, forelimbs and hindlimbs) were scored blindly for abnormalities. Physeal abnormalities were scored: 0 = absent; 1 = present in any long bone; 2 = bilateral symmetry of long bones; 3 = long bones and spine (endplate). Spinal changes were scored: 0 = normal; 1 = 1 disc space narrowed; 2 = >1 disc space narrowed; 3 = 1 space fused and; 4 = >1 disc space fused. Carpal lucency,

hip dysplasia, and stifle effusion seen in an earlier study (23) were not noted. Anterior mitral valve leaflet thickness was measured by 2D echocardiography using right parasternal long axis diastolic views with leaflet tip clearly resolved. Ophthalmology examinations of cornea and anterior chamber used hand-held slit-lamp biomicroscopy (SL-14 slit lamp, Kowa) and binocular indirect ophthalmoscopy. Corneal clouding was scored as absent, mild, moderate or severe by subjective evaluation blind to treatment or affected status. Magnetic resonance imaging of the brain was performed on a 1.5 T GE Signa Excite with standard head coil pre- and post-IV administration of 0.1 mmol/kg gadolinium-DTPA and scored without knowledge of MPS disease or treatment status. Frontal-occipital horn ratio was measured and cortical atrophy scored as described (51). Pre-mortem complete blood counts, serum biochemical concentrations, and cerebral spinal fluid protein concentration, color, and cell counts were performed. ELISA for serum anti-iduronidase IgG antibodies was performed as previously published (12,13), with OD values calculated from dilutions in the linear range. Urinalysis was performed with reagent strips (Bayer Multistix 10 SG). Body weights were obtained weekly.

Statistics

Means and standard deviations were calculated in standard fashion. Multiple comparisons used ANOVA and Tukey-Kramer or Dunnett's *post-hoc* test with $p < 0.05$ considered significant. Total radiograph scores were assessed as described (44), using a general linear mixed model with treatment status and sex as fixed class variables, and age at radiography as a random variable. Simple comparative analysis used 2-tailed Student's *t*-test. Analyses used SAS and SYSTAT statistical software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Enrollment and induction of immune tolerance to IDU. (A) Study enrollment timeline for animals receiving 0.58 mg/kg weekly rhIDU (n=8), indicating age at first rhIDU dose (days) and age at sacrifice (weeks). Animals receiving concomitant intrathecal (IT) ERT are indicated with *. IT ERT was administered at a dose of 0.058 mg/kg (maximum 1 mg) at three-month intervals. (B) Enrollment timeline for dogs treated with 1.57 mg/kg weekly IV rhIDU (n=4). (C) Serum anti-iduronidase IgG antibodies measured by ELISA, depicting pretreatment and final levels and expressed as OD units per μ l undiluted serum on a semi-log scale. Tolerance is defined as an IgG titer of less than 20 OD units/ μ l. Positive non-tolerant control shown for comparison (12,13,21).

Fig. 2.

Glycosaminoglycan (GAG) storage in tissues in dogs treated with IV rhIDU from birth. (A–F) Glycosaminoglycan concentrations measured by Alcian blue dye-binding assay in kidney medulla, kidney cortex, liver, spleen, lung and myocardium of normal dogs, MPS I dogs treated neonatally with 1.57 or 0.58 mg/kg weekly IV rhIDU, and untreated MPS I dogs. Each dot represents one animal. Means and standard deviations were compared by ANOVA. Pairwise post-hoc analysis involved the Tukey-Kramer adjustment and p values are indicated at the top of each graph.

Fig. 3.

Effects of IV rhIDU administration beginning at birth on cardiac disease in MPS I dogs. (A) The GAG storage in the canine mitral valve in normal dogs, MPS I dogs receiving 1.57 mg/kg or 0.58mg/kg weekly IV rhIDU, and untreated MPS I dogs. P-values show comparison of treatment group means to the mean of untreated MPS I dogs (ANOVA with Tukey-Kramer post-hoc analysis). (B) Average thickness (mm) of the anterior mitral leaflet measured by 2-D echocardiography in diastole. P-values show comparison of treatment group means to the mean of untreated MPS I dogs. Each reading is the average of 3 to 15 measurements per dog. (C-E) Images of mitral valve by echocardiography in (C) a normal dog, (D) a 0.58 mg/kg treated MPS I dog, and (E) an untreated MPS I dog. A normal electrocardiogram trace is seen at the bottom of each panel. (F-I) Histopathology of the heart valve (right atrioventricular valve). Black bar indicates 10 μm. (F) Normal, (G) MPS I affected animal treated with 1.57 mg/kg weekly IV, (H) MPS I affected animal treated with 0.58 mg/kg weekly IV, with mild residual lysosomal (GAG) storage indicated by arrowheads, and (I) an untreated affected MPS I dog (lysosomal storage indicated by arrowheads).

Fig. 4.

Effects of IV rhIDU from birth on skeletal abnormalities in MPS I dogs (A–I). Treated dogs averaged 12.8 ± 0.2 months (1.57 mg/kg), or 17.1 ± 1.0 months (0.58 mg/kg) of age, affected dogs 18.1 ± 1.0 months, and normal dogs 18.4 ± 3.2 months. (A–C) Radiographs of normal, (D–F) 1.57 mg/kg treated and (G–I) 0.58 mg/kg treated MPS I affected canines, and (J–L) untreated MPS I affected canines. C3 in (B, E, H, K), the third cervical vertebra. A physal remnant/open physis (circled) is seen in an untreated 17 month old animal (J). (K) Severe narrowing of the intervertebral disc space (circled) in an untreated, affected dog and moderate intervertebral space narrowing in a 0.58 mg/kg weekly treated dog (H), not seen in (B), a normal dog, or (E), a 1.57 mg/kg/wk treated dog. Also evident in (K) is C2–C3 narrowing of the intervertebral space. (L) Toe splaying seen in untreated animal, but not seen in normal or treated dogs (C, F, and I). (M–O) Scoring of skeletal disease indicating average group (M) physis, (N) vertebra, and (O) total scores (see scoring in materials and methods). The two untreated animals depicted in this graph received the same score, resulting in a lack of error bars. Pairwise P values are indicated in the total score bar graph.

Fig. 5.

Effects of IV rhIDU from birth on rib periosteum, joints, skull morphology, joint laxity, and cornea. (A–C) Histopathology of rib periosteum. (D–F) Histopathology of synovium around patella. Untreated affected MPS I (C and F) shows storage of GAGs in both of these tissues, as evidenced by the distended cells. Reduced storage is shown in treated animals (A–B, D–E), with no storage evident in the 1.57 mg/kg treated animals (A, D). (G–I) Skull morphology and (J–L) carpal laxity of normal dogs (G and J), MPS I affected animals receiving 1.57 mg/kg (H and K), and untreated MPS I affected dogs (I and L). Signs of skull abnormalities include a dome-shaped head, a pronounced stop (depressed nasal bridge) and an upturned muzzle (I). (M–O) Hematoxylin and eosin staining of the cornea from a normal dog (M), a dog treated with 1.57 mg/kg (N), and an untreated MPS I dog (O). Intracellular storage in the corneal stroma appears pale and foamy.

Fig. 6.

Effects of IV and IT rhIDU on the brain in MPS I dogs. (A) Magnetic resonance imaging of the brain (T1-weighted with contrast enhancement) in untreated MPS I dog, showing meningeomas (arrows, A). (B–C) Low power (10x, B, Bar = 200 μ m) and high-power (40x, C, bar = 20 μ m) magnification of a fibrous meningeoma in the same untreated MPS I dog. Foamy interstitial cells are indicated by arrows. Lymphoplasmacytic aggregates within the mass are indicated by an arrowhead. (D–F) Toluidine blue staining of cortical neurons in MPS I dogs. Untreated (D), 1.57 mg/kg IV treated (E) and IV/IT treated (F), with lipid granules indicated by arrows. (G–H) Histograms showing glycosaminoglycan concentrations (G) and iduronidase activity (H) in brain of normal, treated MPS I dogs, and untreated MPS I dogs. For iduronidase activity (H), a log₁₀ scale is used for the y-axis. Samples from each of six coronal sections were averaged for each dog, and these means were then averaged for all dogs in the group. ANOVA with post-hoc Tukey-Kramer analysis was used to determine p-values.

Iduronidase enzyme activity in somatic tissues. Iduronidase activity was measured in tissue homogenates with a fluorometric assay, and the means and standard deviation (SD) of the means for each animal calculated.

Table 1

Tissue	Treatment				Normal	
	1.57 mg/kg (n=4) (U/mg protein)		0.58 mg/kg (n=8) (U/mg protein)		(n=5) (U/mg protein)	
	Mean	SD	Mean	SD	Mean	SD
Liver	767	64.8	430	53.8	4.44	1.51
Spleen	72.9	34.1	50.4	21.4	1.96	2.51
Heart valve – Mitral	3.83	2.76	1.11	1.80	6.04**	8.22
Myocardium	0.764	0.221	0.410	0.126	1.76	1.26
Rib Cartilage	7.85	2.77	1.46	1.04	2.48	2.04
Kidney-cortex	33.8	8.98	9.38	2.13	2.33	1.68
Kidney-medulla	35.1	8.51	11.2	3.37	4.55	3.01
Lung	7.53	3.16	2.75	1.23	1.28	1.07

Normal activities are taken from five heterozygous carrier animals from a published study (13). For mitral valve, three carrier animals were available (**).