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Research report

Characterization of an immunodeficient mouse model of mucopolysaccharidosis type I suitable for preclinical testing of human stem cell and gene therapy

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Abstract

Mucopolysaccharidosis type I (MPS-I or Hurler syndrome) is an inherited deficiency of the lysosomal glycosaminoglycan (GAG)-degrading enzyme α-L-iduronidase (IDUA) in which GAG accumulation causes progressive multi-system dysfunction and death. Early allogeneic hematopoietic stem cell transplantation (HSCT) ameliorates clinical features and extends life but is not available to all patients, and inadequately corrects its most devastating features including mental retardation and skeletal deformities. To test novel therapies, we characterized an immunodeficient MPS-I mouse model less likely to develop immune reactions to transplanted human or gene-corrected cells or secreted IDUA. In the liver, spleen, heart, lung, kidney and brain of NOD/SCID/MPS-I mice IDUA was undetectable, and reduced to half in heterozygotes. MPS-I mice developed marked GAG accumulation (3–38-fold) in these organs. Neuropathological examination showed GM_3 ganglioside accumulation in the striatum, cerebral peduncles, cerebellum and ventral brainstem of MPS-I mice. Urinary GAG excretion (6.5-fold higher in MPS-I mice) provided a non-invasive and reliable method suitable for serially following the biochemical efficacy of therapeutic interventions. We identified and validated using rigorous biostatistical methods, a highly reproducible method for evaluating sensorimotor function and motor skills development. This Rotarod test revealed marked abnormalities in sensorimotor integration involving the cerebellum, striatum, proprioceptive pathways, motor cortex, and in acquisition of motor coordination. NOD/SCID/MPS-I mice exhibit many of the clinical, skeletal, pathological and behavioral abnormalities of human MPS-I, and provide an extremely suitable animal model for assessing the systemic and neurological effects of human stem cell transplantation and gene therapeutic approaches, using the above techniques to measure efficacy.
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1. Introduction

MPS-I (Hurler syndrome) is an inherited metabolic disorder due to lack of the lysosomal GAG-degrading enzyme α-L-iduronidase (IDUA) [25]. The resulting GAG accumulation causes multi-system dysfunction and death within the first decade. One of the most devastating manifestations of Hurler syndrome occurs in the central nervous system (CNS), resulting in progressive neuro-psychological deterioration [21,37].

Novel approaches such as gene therapy and stem cell-based therapy hold great potential for development into clinically

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useful interventions. Pre-clinical testing of these modalities requires the availability of animal models that consistently and accurately represent the human disease. Several such animal models include the MPS-I mouse [5,27,29,38], domestic cat [13,14] and Plott hound dog [31,33,35]. A major limitation of the above models is that they are not immunodeficient, and can develop immune reactions against transplanted human or gene-corrected cells and/or the secreted enzyme [6,10,17,20,24,32]. Furthermore, evaluation of novel CNS-based approaches will require a test of neurological function that is easy to perform serially and consistently measures neurological deficits relevant to the human disease.

The two currently available murine models of MPS-I [5,27,29,38] were generated by insertion of a neomycin resistance gene in exon 6 of the IDUA gene in the background of the C57BL/6 strain, and both are immunocompetent.

$IDUA^{null}$ mice developed by Clarke et al. [5,29] lack detectable tissue enzyme activity, exhibit a 2–5-fold increase in urinary GAG excretion, progressive lysosomal accumulation of GAGs in various organs including the brain and progressive skeletal histopathology and dysostosis with visible broadening of facial features and digits [5]. These mice demonstrate abnormal growth and shortened life span (average survival 48 weeks), progressive Purkinje cell loss in the cerebellum, and increased accumulation of GM_2 and GM_3 gangliosides in the brain [29]. Cardiovascular abnormalities include aortic root dilatation, aortic valve insufficiency, increased left ventricular size and decreased left ventricular function [3]. Neurological function has not been reported on for this model.

The $IDUA^{null}$ mice developed by Neufeld and co-workers [27,38] have a similar but not identical phenotype as the Clarke model. Cardiovascular abnormalities include enlargement of the heart with thickening of the septal and posterior walls and reduced left ventricular function, thickening and distortion of the valves with insufficiency of the aortic and mitral valves, electrocardiographic abnormalities, disturbed circadian rhythm and aortic dilatation [16,19]. Distinct craniofacial skeletal abnormalities have been identified by computed tomographic scanning [11]. These mice have reduced hearing and photoreceptor function and fewer cells in the outer nuclear photoreceptor layer of the retina [19,22]. Behavioral abnormalities detected include abnormal habituation in the open field test [12,28] and impairment of long-term (but not short-term) memory for inhibitory avoidance training, whereas novel object recognition memory and nociception are normal [28].

We have characterized an immunodeficient mouse model of MPS-I [the NOD.129(B6)-*Prkdc^scid^Idua^tm1Clk^* mouse] that breeds well under the conditions described here and demonstrates neurological and systemic manifestations of the disease. Further, we have validated that the Rotarod test represents an easily performed and consistent measure of impaired sensorimotor function and development of motor coordination in this mouse model. The NOD/SCID/MPS-I mouse thus provides a suitable model for evaluating the potential benefits of human stem cell, gene or enzyme-based therapies.

2. Methods

2.1. Animals

All studies were approved by the University of Minnesota's Institutional Animal Care and Use Committee (IACUC). Breeder pairs of NOD.129(B6)-$Prkdc^{scid}Idua^{tm1Clk}$ mice heterozygous for the IDUA mutation were obtained from The Jackson Laboratory (Bar Harbor, ME), housed under specific-pathogen-free (SPF) conditions and provided with chow (TEKLAD #2018), sterile/acidified water and sulfmethoxazole-trimethoprim antibiotics biweekly. A total of 24 breeder pairs were set during the characterization of the colony (different combinations of the same mice within the colony). On average, a WT × WT mating produced 9 offspring whereas a heterozygous × heterozygous mating produced an average of 7 offspring. Attempts to mate MPS-I females with heterozygous males were unsuccessful. The colony was therefore propagated by heterozygous × heterozygous mating. This produced approximately 25% homozygous NOD-SCID-MPS-I ($IDUA^{null}$:MPS-I) mice, 50% heterozygous ($IDUA^{heterozygous}$:Het) carriers and 25% wild type NOD-SCID mice ($IDUA^{normal}$:WT). The average litter size was 7, with 53% female and 47% male offspring produced.

We initially observed that mothers often cannibalized their offspring during the first 1–4 days. Provision of a mix of peanut butter and oatmeal to the parents, and rubbing a small quantity of Vicks Vaporub® on the mothers' snouts and on the offspring were remarkably successful in reducing cannibalization.

2.2. Genotyping of mice

Tail clippings were digested overnight at 55 °C in 200 μg/ml proteinase K in lysis buffer (1% SDS, 0.3 M sodium acetate, 10 mM Tris and 1 mM EDTA). Samples were frozen at −80° C for at least 30 min, then clarified by centrifugation at 14,000 rpm at 4 °C for 15 min. The supernatant was removed to a new tube, clarified again by centrifugation, and 2 μl was used for genotyping using PCR performed according to the protocol on The Jackson Laboratory website: (http://jaxmice.jax.org/pub-cgi/protocols/protocols.sh?objtype=protocol&objopr=query&protocol_id=466&no_rxns=20&button=Calculate). The following primers were used at a final concentration of 120 nM each:

Forward oIMR 1451: (5′-GGAACTTTGAGACTTGGAATGAACCAG-3′).
WT reverse oIMR 1452: (5′-CATTGTAAATAGGGGTATCCTTGAACT-3′).
MPS reverse oIMR 1453: (5′-GGATTGGGAAGACAATAGCAGGCATGCT-3′).
PCR products were resolved on a 1.5% agarose gel.

2.3. Photography of mice

Female and male mice at 6 months of age were sedated, placed on a black background and photographed using a 2.0 megapixel digital camera.

2.4. Skeletal radiographs

Eight month old mice were sedated and whole body radiographs obtained using a Faxitron (MNR 2000 Screen; exposure 7 s/24 kV).

2.5. Rotarod test for sensorimotor and behavioral testing

2.5.1. Experimental method

A rotating rod apparatus (Model 8200 LSi Letica, PANLAB, Spain) was used to measure the ability of WT, heterozygous and MPS-I mice to remain balanced on a rotating rod (5 cm diameter) at different speeds. Mice used in this experiment were 20 weeks of age and naïve to this test. A total of 14 females (4 WT, 6 heterozygous and 4 MPS-I) and 21 males (6 WT, 9 heterozygous and 6 MPS-I) were tested. Mice were placed on the rod that was rotated at incremental speeds of 5, 15, 25 or 35 rpm three times daily for 5 consecutive days. Latency to fall (in seconds) was scored for each animal at each speed in each trial. Animals rested for approximately 20 min between trials to prevent fatigue and

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