BRAIN RESEARCH 1188 (2008) 241–253

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Progression of multiple behavioral deficits with various ages of onset in a murine model of Hurler syndrome

Dao Pan[a,c,*], *Anthony Sciascia II*[a], *Charles V. Vorhees*[b,c], *Michael T. Williams*[b,c]

[a]*Molecular and Gene Therapy Program, Division of Experimental Hematology, Cincinnati Children's Research Foundation, Cincinnati, OH, USA*
[b]*Division of Neurology, Cincinnati Children's Research Foundation, Cincinnati, OH, USA*
[c]*Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, USA*

ARTICLE INFO

Article history:
Accepted 17 October 2007
Available online 23 October 2007

Keywords:
Animal model
Lysosomal storage disease
Hurler syndrome
Behavior profile
Spatial learning
Open-field
Locomotion

ABSTRACT

Mucopolysaccharidosis type I (MPS I) is one of the most common lysosomal storage diseases with progressive neurological dysfunction. To characterize the chronological behavioral profiles and identify the onset of functional deficits in a MPS I mouse model (IDUA$^{-/-}$), we evaluated anxiety, locomotor behavior, startle, spatial learning and memory with mice at 2, 4, 6 and 8 months of age. In automated open-field test, IDUA$^{-/-}$ mice showed hypoactivity as early as 2 months of age and altered anxiety starting from 6 months of age during the initial exploratory phase, even though normal habituation was observed at all ages. In the marble-burying task, the anxiety-like compulsive behavior was normal in IDUA$^{-/-}$ mice at almost all tested ages, but significantly reduced in 8-month old male IDUA$^{-/-}$ mice which coincided with the rapid death of IDUA$^{-/-}$ males starting from 7 months of age. In the Morris water maze, IDUA$^{-/-}$ mice exhibited impaired proficient learning only at 4 months of age during the acquisition phase. Spatial memory deficits were observed in IDUA$^{-/-}$ mice during both 1 and 7 days probe trials at 4 and 8 months of age. The IDUA$^{-/-}$ mice performed normally in a novel object recognition task at younger ages until 8 months old when reduced visual cognitive memory retention was noted in the IDUA$^{-/-}$ mice. In addition, 8-month-old IDUA$^{-/-}$ mice failed to habituate to repeated open-field exposure, suggesting deficits in non-aversive and non-associative memory. In acoustic startle assessment, significantly more non-responders were found in IDUA$^{-/-}$ mice, but normal performance was seen in those that did show a response. These results presented a temporal evaluation of phenotypic behavioral dysfunctions in IDUA$^{-/-}$ mice from adolescence to maturity, indicating the impairments, with different ages of onset, in locomotor and anxiety-like compulsive behaviors, spatial learning and memory, visual recognition and short-term non-associative memory retention. This study would also provide guidelines for the experimental designs of behavioral evaluation on innovative therapies for the treatment of MPS type I.

* *Corresponding author*. Molecular and Gene Therapy Program, Division of Experimental Hematology, Cincinnati Children's Research Foundation, 3333 Burnet Ave., Cincinnati, OH 45249, USA. Fax: +1 513 636 1333.
E-mail address: dao.pan@cchmc.org (D. Pan).

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.10.036

1. Introduction

Mucopolysaccharidosis type I (MPS I) is one of the most common lysosomal storage diseases (LSD). As a group, LSDs have a collective incidence of ~1 in 7000 live births and 65% of these affect the central nervous system (CNS) (Neufeld, 1991). MPS I is caused by the deficiency of alpha-L-iduronidase (IDUA) and the subsequent systemic accumulation of glycosaminoglycans (GAGs). The clinical features in patients with MPS I are associated with progressive systemic tissue pathology and multi-organ dysfunctions. In severe forms of MPS I (i.e., Hurler syndrome), the CNS complications are devastating and characterized by hydrocephalus, learning delays and mental retardation culminating in dementia. If untreated, children with MPS I usually die before the age of 10. As one of the current treatments for MPS I, allogeneic bone marrow transplantation (BMT) is effective in prolonging a child's life and ameliorating many of the clinical manifestations of the disease (Church et al., 2007; Krivit et al., 1999). However, clinical benefits of BMT for CNS abnormalities vary dramatically and seem to be associated with the age of treatment. For example, when children with MPS I are treated with allogeneic BMT early in life (less than 2 years old), there is an attenuation of CNS deterioration or in some cases preservation of normal intellectual function (Peters et al., 1996; Shapiro et al., 1995). In contrast to early BMT therapy, little benefit was shown if CNS deterioration was apparent prior to treatment (Souillet et al., 2003; Staba et al., 2004). Thus, optimal prevention of CNS deterioration in MPS is possible only when intervention (treatment) occurs before the onset of neurological dysfunction. Therefore, early diagnosis and determination of the optimal timing for intervention or a window for treatment could be crucial to successful therapy. However, in the human literature, there are only a few detailed case history studies on clinical outcomes or disease progression that address CNS aspects of the neuronopathic forms of LSD and in particular MPS I. In addition, because of the limitation of BMT with the risk of significant mortality and the high rate of engraftment failure (Schiffmann and Brady, 2002), innovative therapies with consistent long-term clinical benefits are still needed.

A transgenic *IDUA* knockout murine model (IDUA$^{-/-}$, or MPS I), generated by disruption of the open reading frame with an insertion in exon 6 (Clarke et al., 1997), has made it possible for the systematic evaluation of the neurological deficits associated with MPS I. In this regard, some behavioral impairments have been reported by us (Hartung et al., 2004; Pan et al., 2003) and others (Reolon et al., 2006) using this mouse model. We found that animals subjected to 3 consecutive short-term open field tests, 5 min each with an intertrial interval of 30 (Pan et al., 2003) or 90 (Hartung et al., 2004) min, failed to habituate to the testing apparatus (i.e., showed sustained activity) compared to normal animals on the third trial. In the study by Reolon et al. (2006), IDUA$^{-/-}$ mice performed similarly to controls during a 5 min open-field test including the number of line crossings and latency to begin moving after being placed in the apparatus, except that IDUA$^{-/-}$ mice showed a reduced number of rears compared to controls. Furthermore, no differences were observed in a novel object recognition task or in an inhibitory step-down avoidance task with a 90 min retention interval. However, when animals were examined 24 h after inhibitory avoidance training, the IDUA$^{-/-}$ mice showed deficits in memory. Taken together, these data suggest that the IDUA$^{-/-}$ mice have an altered locomotor response to an open-field upon multiple presentations and have some degree of memory inhibition, suggesting this mouse is representative of some defects seen in patients with MPS I (i.e., Hurler syndrome).

While the previous data identified some specific phenotypes of IDUA$^{-/-}$ mice compared to controls, the progressive deterioration of CNS function with age has not been addressed. In fact, the previous studies examined animals only at a single time point (4 months old by us or 5–7 months mixed age by Reolon), and also did not consider potential sex differences. These considerations are especially important because the progressive nature of the disease, gender differences, complications arising from other symptoms (e.g., musculoskeletal dysfunctions) on behavioral appearance or the combination of these may influence behavioral outcomes. Therefore, a detailed evaluation is needed to determine when the IDUA$^{-/-}$ mice begin to demonstrate specific behavioral phenotypes (e.g., locomotor changes), if sexual dimorphism exists in the progression of the disease, if other cognitive abilities, such as spatial learning and memory, are affected and if anxiety-related behaviors might also be changed (since altered anxiety can influence learning and memory).

In the present report, we examined IDUA$^{-/-}$ mice repeatedly, every other month from 2 to 8 months of age, in behavioral assessments measuring anxiety, locomotor behavior and spatial learning and memory. These tasks were selected because of previous data and because of the brain pathology associated with IDUA$^{-/-}$ mice. In particular, abnormal lysosomal storage, i.e., cytoplasmic vacuolation, has been found in the neurons of cerebral cortex and Purkinje cells of the cerebellum as early as 2 months of age (Clarke et al., 1997). Abnormal GAG accumulation and/or GM2 ganglioside staining has been demonstrated in the hippocampus, neostriatum, cerebellum and cortical areas as well as other regions in adult MPS I mice (older than 3-months) (Chung et al., 2007; Hartung et al., 2004). Both the Morris water maze and novel object recognition task are known to be dependent upon the hippocampus, and other regions for proficient learning of these tasks (Clark et al., 2000; Squire and Zola, 1996; Zola et al., 2000). In order to determine if sensorimotor deficits existed, we tested animals for their ability to navigate to a cued platform in the Morris water maze at 2 months of age. We also examined animals at 8 months to determine if they were responsive to acoustic startle stimuli since hearing can be affected in IDUA$^{-/-}$ mice (Schachern et al., 2007) and the cerebellum is also involved in acoustic startle. Animals were examined in the repeated open-field habituation test at 8 months of age to confirm our previous results and as a comparison to the automated locomotor tests that we did at all ages. Finally, anxiety-related behavior was examined because the progression of systemic symptoms with age can affect stress levels in MPS I, and changes in anxiety can affect learning and memory tasks. The overall aims of these assessments were to characterize the chronological behavioral profiles in MPS I mice and identify the onset of functional deficits.