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Research report

Behavioural characterisation of the α-mannosidosis guinea pig

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Abstract

α-Mannosidosis is a lysosomal storage disorder resulting from a functional deficiency of the lysosomal enzyme α-mannosidase. This deficiency results in the accumulation of various oligosaccharides in the lysosomes of affected individuals, causing somatic pathology and progressive neurological degeneration that results in cognitive deficits, ataxia, and other neurological symptoms. We have a naturally occurring guinea pig model of this disease which exhibits a deficiency of lysosomal α-mannosidase and has a similar clinical presentation to human α-mannosidosis. Various tests were developed in the present study to characterise and quantitate the loss of neurological function in α-mannosidosis guinea pigs and to follow closely the progression of the disease. General neurological examinations showed progressive differences in α-mannosidosis animals from approximately 1 month of age. Significant differences were observed in hind limb gait width from 2 months of age and significant cognitive (memory and learning) deficits were observed from 3 months of age. Evoked response tests showed an increase in somatosensory P1 peak latency in α-mannosidosis guinea pigs from approximately 2 months of age, as well as progressive hearing loss using auditory brainstem evoked responses. The α-mannosidosis guinea pig therefore appears to exhibit many of the characteristics of the human disease, and will be useful in evaluating therapies for treatment of central nervous system pathology.

Keywords: Lysosomal storage disorder; α-Mannosidosis; Guinea pig; Behavioural test

1. Introduction

α-Mannosidosis is a lysososmal storage disorder (LSD) resulting from a deficiency of the lysosomal enzyme α-mannosidase (E.C. 3.2.1.24; OMIM #248500). This in turn results in the lysosomal accumulation of various macromolecular substrates, with a range of pathological consequences. α-Mannosidosis patients exhibit coarse facial features and hepatosplenomegaly, along with progressive neurological decline resulting in mental retardation and ataxia [2,27].

Although α-mannosidosis in humans and animal models has been treated with bone marrow transplantation [31,32], this approach has shown only limited efficacy for treatment of central nervous system pathology, along with a high risk of morbidity/mortality. Additionally, bone marrow transplantation requires both a suitably matched donor, and early diagnosis and treatment for the best outcome. Direct injection of gene transfer vectors has also been evaluated in the feline α-mannosidosis model, with widespread improvement observed in CNS pathology [30], however further refinements are necessary before it could be used in humans. Accordingly, much research has and continues to focus on treatments for central nervous system pathology in α-mannosidosis and other LSD. These studies require well-characterised animal models to effectively evaluate potential therapies.

α-Mannosidosis has been reported in cattle [18], cats [5,6,20,28], mice [26] and guinea pigs [3,8,23]. The α-mannosidosis guinea pig has a deficiency of lysosomal α-mannosidase, with activity ranging from approximately 2.5% of normal activity in leukocytes, to 23% of normal activity in cultured skin fibroblasts [8]. These guinea pigs display mild

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somatic pathology and significant, progressive neuropathology [8], which first becomes apparent *in utero* [1]. We have an outbred colony of α-mannosidosis guinea pigs, which provides a more clinically relevant model for evaluating therapies and investigating pathology than some inbred strains of mice.

The aim of this study is to further characterise the nature and progression of neurological changes in the α-mannosidosis guinea pig, using a battery of behavioural and neurological tests. All animals except those used for electrophysiology testing were also receiving concurrent Cyclosporine treatment as part of a larger study [25].

Generalised neurological examinations have previously been described for use in small domestic animals [11]. In the present study, we have adapted this examination to identify various signs and symptoms present in humans with this disorder, along with those noted anecdotally in α-mannosidosis guinea pigs. We have also examined the time course of appearance of gross motor changes (by assessing gait width and length), as α-mannosidosis guinea pigs were reported to show signs of ataxia or motor incoordination when they were first described [8]. Cognitive function has been assessed using the Morris water maze [22], a test of memory and learning originally developed for use in rats. Due to the observation that α-mannosidosis cats exhibit decreased nerve conduction velocities [29], and α-mannosidosis patients experience progressive hearing loss as part of the course of their disease [2,27], somatosensory and auditory brainstem evoked responses were also tested.

2. Methods

2.1. Animals

All animal studies were performed with approval from the Children, Youth and Women's Health Service Institutional Animal Ethics Committee and were conducted in accordance with the National Health and Medical Research Council of Australia guidelines. The α-mannosidosis guinea pig colony was originally established from two founder heterozygotes in 1999, and has been maintained as an outbred colony, with new genetic material sourced from external animal suppliers every two to three generations. Guinea pigs were housed in groups of five to eight, in an enriched environment consisting of housing boxes and PVC pipes, with *ad libitum* food and water on a 12-h light:12-h dark cycle at 22 °C, 45–50% humidity.

Genotypes were confirmed using a PCR-based protocol described previously [3]. Both normal guinea pigs and animals heterozygous for the α-mannosidosis mutation are phenotypically normal and are henceforth referred to as normal.

Two groups of animals were used in this study. The first group of guinea pigs were tested using the Morris water maze, gait analysis and neurological examinations. As part of a larger study involving stem cell implantation into the brain [25], these animals (both α-mannosidosis and normal controls) received a sham injection of 1 μl cell culture medium (Dulbecco's Modified Eagle Medium, GIBCO, Invitrogen) *without* stem cells into the dentate gyrus of the hippocampus at 1 week of age, using a stereotaxic frame. They were also weighed and treated daily with Cyclosporine A (CSA, 20 mg/kg orally once daily for 2 weeks commencing at 1 week of age, followed by 10 mg/kg once daily thereafter). The second group of guinea pigs were only used for somatosensory/auditory brainstem evoked response studies, and did not undergo any surgical procedures or Cyclosporine treatment.

2.2. Multiple component neurological examination

Neurological examinations (based on De Lahunta [11]) were performed weekly, with up to six guinea pigs tested at a time. Testing groups included both normal and α-mannosidosis animals. Test components were assigned different maximum scores to reflect the relevance of each component of pathology to disease progression in the α-mannosidosis guinea pig, with some graded according to increasing severity. Scores were then added and each animal was given a total score. Fig. 1 shows the score sheet developed for these tests.

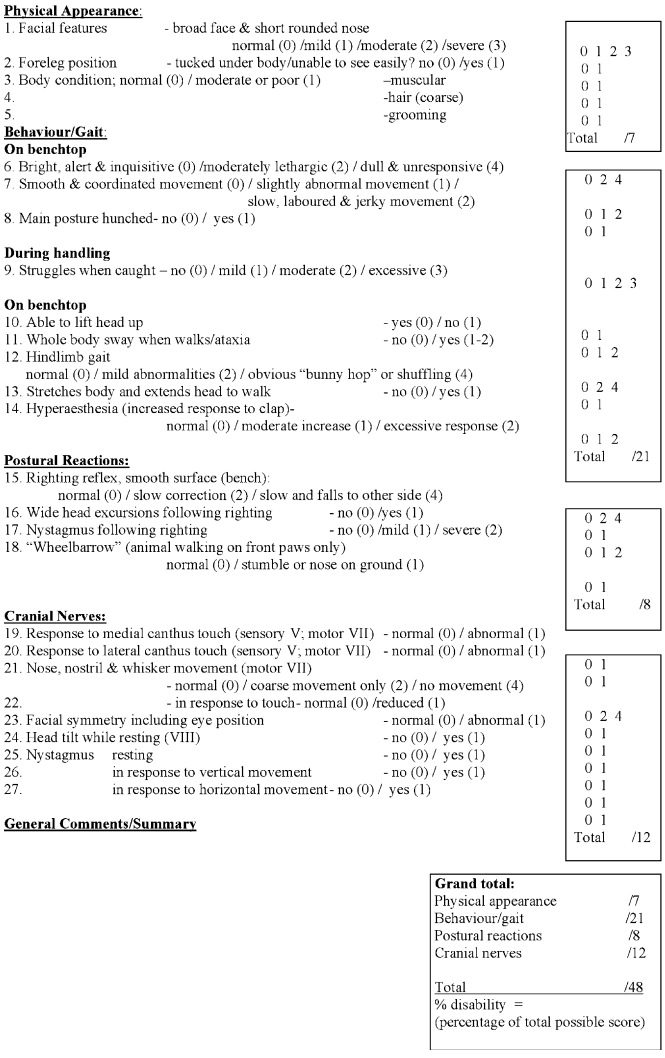

Fig. 1. Guinea pig neurological examination. Numbers in parentheses indicate the score assigned to each animal tested depending on the severity of each neurological feature. Scores are circled for each animal in the column on the right, and totalled where indicated.

The observer was blind to all previous test scores during neurological examinations, but was not blind to animal identity or genotype. All data were collated and analysed after the testing period.

2.3. Gait analysis

Hind limb gait width and length were measured using a method described previously for mice [17]. Briefly, gait was analysed using a long box (approximately 1 m long by 0.3 m wide), high at the sides and open at the top, with a dark enclosure at one end, containing food (apple) as an incentive. Paper for recording gait patterns was placed on the floor of the box. The hind paws of the guinea pigs were dipped in non-toxic food colouring, and the animals were then placed at the open end of the box, such that they would walk along the paper to the dark enclosure. Animals were tested until two clean gait patterns (i.e. animal walking all the way along the paper) were obtained for each animal at each time. Gait length and width were measured manually from the footprints collected. Gait speed was not measured in this study.