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AGE-RELATED FUNCTIONAL AND HISTOPATHOLOGICAL CHANGES OF THE EAR IN THE MPS I MOUSE

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Abstract

Objective— Mucopolysaccharidosis type I (MPS I) is an autosomal recessive disorder caused by a mutation in the gene encoding the enzyme α -L-iduronidase. This enzyme is responsible for degradation of dermatan and heparan sulfates. Enzyme deficiency results in their accumulation in lysosomes of virtually all organs, resulting in severe somatic and neurological changes. Clinical findings of otitis media with mixed hearing loss are common. Cellular and molecular mechanisms of ear pathology and hearing loss are not understood. The purpose of this study is to describe the age-related audiologic and histopathologic changes of the ear in the mouse model of MPS I.

Methods— Auditory brainstem responses (ABR) were obtained to clicks and tone bursts at 1-32 kHz, and pathological changes to middle and inner ears were studied with light and electron microscopy in fifty-three mice that included: 1) wild type (+/+) - five at 2 months, five at 4 to 6 months, and five at 13 to 19 months; 2) heterozygotes (+/-) - four at 2 months; five at 4 to 6 months; and eight at 13 to 19 months; and 3) homozygotes (-/-) - five at 2 months; six at 4 to 6 months; and five at 13 to 19 months. Histopathology was also done on five newborn -/- mice.

Results— In newborns no lysosomal storage was observed and the ear appeared age appropriately normal. In all other -/- mice, cells with lysosomal storage vacuoles were observed in spiral ligament, spiral prominence, spiral limbus, basilar membrane, epithelial and mesothelial cells of Reissner's membrane, endothelial cells of vessels, and some ganglion cells; their number increased with aging. Hair cell loss was not observed at 2 or 6 months, but there was total loss of the organ of Corti in year-old mice. Hearing of -/- mice was significantly decreased at all ages compared to +/+ and +/-.

Hearing loss progressed from mild to moderate loss at 2 months to profound at 6 months and total deafness by one year of age.

Conclusions— Progressive age-related changes suggest early therapeutic intervention to prevent sensory cell damage and hearing loss.

Keywords

MPS I mouse; middle ear; cochlea; hearing loss; ear pathology; auditory brainstem response

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INTRODUCTION

Mucopolysaccharidosis type I (MPS I) is one of several variants of lysosomal storage diseases that involve the accumulation of various mucopolysaccharides. It is an autosomal recessively inherited disorder caused by a mutation in the gene encoding the enzyme α -L-iduronidase [1]. This enzyme is responsible for the degradation of dermatan sulfate and heparan sulfate glycosaminoglycans (GAGs) and its deficiency results in accumulation of undegraded GAGs in lysosomes. Due to the wide distribution of GAGs in the body, virtually all organs are affected resulting in severe somatic and neurologic changes. Otolaryngological manifestations include recurrent middle ear effusions [2,3] and progressive mixed hearing loss [2]. Murine models of MPS, deficient in specific enzymes are available. Functional [4] and histopathological changes [4,5] of the ear have been described for the murine model of MPS VII. Liu et al. [6] described reduced hearing of the MPS I mouse at 8 months of age. To our knowledge, no one has studied the age-related functional and histopathological changes in the ears of a murine model of MPS I. The aim of this study was to determine functional and histopathological changes with ageing.

MATERIALS AND METHODS

Fifty-three mice, newborn to 19 months of age were included in this study. For $+/+$ mice, there were five at 2 months, five at 4 to 6 months, and five at 13 to 19 months of age. For $+/-$ mice, there were four at 2 months; five at 4 to 6 months; and eight at 13 to 19 months of age. For $-/-$ mice, there were five newborn; five at 2 months; six at 4 to 6 months; and five at 13 to 19 months of age. The care and use of animals used in this study were approved by the Institutional Animal Care and Use Committee of the University of Minnesota. All animals were anesthetized intraperitoneally with a combination of ketamine (40mg/kg) and acepromazine (0.5 mg/kg) prior to auditory brainstem response (ABR) recordings (supplemental doses, 30 % of the initial amount, were administered at intervals of 45 min). Animals were anesthetized again, prior to sacrifice. Newborn animals did not have auditory testing.

Auditory Brainstem Response

Body temperature was actively maintained at 37° C by placing the animal on a thermal blanket and keeping the test room at a high ambient temperature. ABRs were collected by differentially amplifying voltages presented at subdermal needle electrodes using a standard vertex positive, ipsilateral mastoid-negative montage. ABR recordings, in response to calibrated acoustic signals, were obtained in mice to verify auditory sensitivity to rarefaction clicks and tone bursts over a 1–32 kHz range. Total tone durations were from 1 to 4 ms and consisted of Blackman rise/fall times and no plateau. Five hundred twelve presentations were averaged at each intensity. The gain of the physiologic amplifier was set to 100,000X for ABRs. Threshold was determined in 5 dB steps of decreasing stimulus intensity, until waveforms lost reproducible morphology. Analysis of variance (ANOVA) was used to test any differences of ABR thresholds using SPSS software (SPSS Inc, IL). All data were expressed as the mean $\pm$ standard error of mean. Differences in thresholds between IDUA $-/-$, $+/-$ and $+/+$ mice were analyzed using the non-parametric Wilcoxon rank sum test. Statistical significance was defined as $P < 0.05$. Following ABR, mice were decapitated and the left and right bulla removed. One side was processed for light microscopy and the other for electron microscopy.

Histopathology

Paraffin embedding—The bulla was opened and filled with 4% paraformaldehyde. Fixation was continued by emersion for 2 hours. Samples were decalcified in 10% EDTA on a rotator in a cold room for 3 days. EDTA was changed daily. Samples were dehydrated in a graded series of ethanol, followed by two changes in xylene, and embedded in paraffin. Sections were cut at a thickness of 5 μ m and stained with hematoxylin and eosin.

Epoxy embedding—The bulla was opened and the cochlea perfused via the oval and round windows with 2% glutaraldehyde in 0.1M phosphate buffer (pH 7.4). Cochleae were not perfused in newborn mice. Fixation was continued by emersion for 2 hours. Samples were decalcified in 10% EDTA on a rotator in a cold room for 3 days. EDTA was changed daily. Samples were washed in phosphate buffer and post-fixed in 1% OsO₄ in phosphate buffer (pH 7.4) for 1 hour. They were washed again in buffer, dehydrated in a graded series of ethanol, followed by propylene oxide, and embedded in epoxy resin. Samples were cut at a thickness of 1 µm and stained with Toluidine blue for light microscopic assessment. For electron microscopy, samples were cut at a thickness of 20 nm, stained with uranyl acetate and lead citrate, and examined with a JEOL 100S electron microscope.

RESULTS

Auditory Brainstem Response

Hearing thresholds of +/+, +/-, and -/- mice at 2 months of age were 30 dB (SE: 1.8), 31.8 dB (SE: 3.5), and 55.8 dB (SE: 3.7), respectively (Fig. 1a). There was a significant difference between the -/- group and the other groups. Hearing thresholds of +/+, and +/- mice at 6 months of age were 34 dB (SE: 2.3) and 37 dB (SE: 2.3) respectively. In -/- mice at 6 months of age, hearing threshold was elevated at frequencies of 12 kHz and 16 kHz; however, mice were totally deaf above and below these frequencies (Fig. 1b). Hearing thresholds of +/+ and +/- mice at one year of age were 54.3 dB (SE: 2.8) and 51.8 dB (SE: 1.9), respectively (Fig. 1c). No hearing was detected at any frequency in the -/- group at one year of age. There was a significant difference between the -/- group and the other groups at all ages and frequencies (Fig. 1a–c).

Histopathology

Findings in the +/+ and +/- animals were similar at all ages. Otitis media was not observed at any age. Organs of Corti and stria vascularis appeared normal in the 2 and 6-month animals. At one year of age, there were occasional missing outer hair cells. In the -/- mice, otitis media was observed at 2 months, 6 months and one year of age (Fig. 2). Cells containing lysosomal granules and vacuoles were not observed in the inner ears of newborn mice. Cell containing lysosomal granules and vacuoles were observed in mice at two months of age in the area of the Type I, II, III and IV fibrocytes of the spiral ligament (Fig. 3). The area of the type IV fibrocytes was affected most and Type III least. Cells containing lysosomal storage were also seen in the spiral prominence, spiral limbus (Fig. 4), mesothelial cells lining the basilar membrane (Fig. 5), both epithelial and mesothelial cells of Reisner's membrane (Fig. 6 a,b), endothelial cells of vessels, and some ganglion cells (Fig. 7). Cell with lysosomal storage granules were not observed in the stria vascularis or hair cells (Fig. 8a,b) at any age. At two months of age, the hair cells and stria vascularis appeared normal. Findings in animals at six months of age were similar to those at 2 months, but with an increase in lysosomal vacuoles. In all animals at one year of age or older, the organs of Corti and neurofilaments were missing, however, the morphology of the stria vascularis appeared normal (Fig. 9 a,b).

DISCUSSION

Mucopolysaccharidosis type I is the most severe form of the family of lysosomal storage disorders. Most organs of the body are affected with severe mental retardation and death in childhood. Otitis media is a universal problem with this disease and is often a presenting symptom [2,3]. Clinical [2,3] and histopathological findings [7–9] show a mixed hearing loss, however, mental retardation, due to changes of the cerebral cortex, often confounds hearing testing, and the pathological component behind the sensorineural loss is not known. Mouse

models of this disorder may give some insight into the histopathological correlates of sensorineural hearing loss.

Functional and histopathological findings of the ear have been reported in a murine model of MPS VII. Mucopolysaccharidosis VII is caused by a deficiency of β -glucuronidase [10]. It is a very rare form of mucopolysaccharidosis, which shares many of the same, but milder clinical features as MPS I including hearing impairment [11]. Ohlemiller, et al. [4] studied the functional and histopathological changes of the MPS VII murine model from 1.0 to 7.5 months of age. Although hearing thresholds were abnormal, they found no evidence of cell loss within the organ of Corti. Berry et al. [5] found some focal loss of hair-cell stereocilia in young adults with damage increasing with age in older animals with MPS VII. In our study of the MPS I mouse, we observed aged-related pathological changes both in the middle and inner ears. We found no cochlear cell loss at 6 months of age, but total loss of the organ of Corti was seen in the 12-month mice. The difference in our pathological findings of ageing animals compared to the MPSV II model could be because MPS I is the most severe form of the MPS storage diseases. Another reason may be because we observed a progression of disease with aging, and the oldest animals in our study were more aged than those in the MPS VII studies. Berry et al. [5] described lysosomal distension in inner hair cells; however, Ohlemiller et al. [4] did not observe lysosomal granules in any hair cells in animals with MPS VII. We also did not find lysosomal granules in hair cells, indicating damage to the hair cells might be secondary to the lysosomal storage and degeneration of other cells.

Fibrocytes of the spiral limbus and spiral ligament have been reported to express carbonic anhydrase and Na,K-ATPase [12], connexins [13], Na-K-Cl co-transporter [14], and chloride-iodide transport protein [15] and are, thus, thought to play a significant role in maintaining the ion homeostasis of the cochlea. In a study of C57 BL/6 mice, Hequembourg and Liberman [16] found that Type IV fibrocytes degeneration preceded loss of hair cells and neurons in both space and time. They found a hearing threshold shift in 7-month animals that did not have hair cell loss and attributed this shift to reduction in the endolymphatic potential resulting from loss of Type IV fibrocytes. Delprat et al. [17] discovered otospiralin, an ear specific protein produced by fibrocytes of the spiral limbus and spiral ligament. They found that down-regulation of otospiralin resulted in rapid and severe degeneration of the organ of Corti leading to irreversible deafness. In our 6-month $-/-$ animals, there was a profound hearing loss with no loss of cochlear hair cells, but with damage and loss of fibrocytes in the lateral cochlear wall. This was followed at 12 months by total loss of the organ of Corti and neurons. Given the variety of important functions of these fibrocytes, related to the maintenance of the ionic composition of the endolymph and perilymph, it may be that damage of the hair cells in MPS I mice occurred secondary to the damage of these fibrocytes. The high level of lysosomal storage granules in cells of Reissner's membrane, which separates the endolymph from the perilymph, may also cause disturbance of ion and fluid homeostasis in the cochlea contributing to the loss of sensory cells.

In our study, the changes we observed in this model were progressive overtime and included: 1) ears that appeared age appropriately normal at birth; 2) lysosomal storage with mild hearing loss and retention of sensory cells at 2 months; 3) lysosomal storage with a profound hearing loss and retention of sensory cells at 6 months; and 4) lysosomal storage with total loss of both hearing and sensory cells at one year. Liu et al. [6] studied retroviral vector-based gene therapy of neonatal MPS I mice and found that those mice that received high dose therapy at birth had near-normal hearing at 8 months of age compared to non-treated mice who had profoundly reduced hearing. We have observed therapeutic responses in other organs of newborn mice treated by AAV and lentiviral gene therapy (18,19). These findings suggest that early diagnosis and therapeutic intervention are critical to the prevention of sensory cell damage and hearing loss in patients with this disorder. Since newborn screening of MPS is currently not accepted

practice, target screening of a high-risk population should identify many affected individuals (20). In our study of human temporal bones from patients with MPS I, we found residual fetal mesenchyme filling the middle ears of all temporal bones, even in patients as old as 6 years of age [7]. This suggests that these children would likely not pass newborn hearing screening. We recommend testing every child who fails newborn hearing screening for MPS disease.

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Figure 1.

Comparison of ABR thresholds in mice: a) 2 months of age; b) 6 months of age; and c) 12 months of age. Hearing of $-/-$ mice was significantly decreased at all ages compared to $+/+$ and $+/-$. Hearing loss progressed from mild to moderate loss at 2 months to total deafness by 12 months.

Figure 2.

Otitis media was seen in $-/-$ mice at all time periods. In this animal at 1-year of age, middle ear effusion (arrow) and granulation tissue (G) can be seen in the middle ear. Staining with Hematoxylin and eosin. Original magnification X20.

Figure 3. Lysosomal granules (arrowheads) and vacuoles (arrows) can be seen in the type IV fibroblasts of the spiral ligament (SL) in this one-year-old $-/-$ mouse. Staining with Toluidine blue. Original magnification: X1,000.

Figure 4.
There are lysosomal granules (arrowheads) in the spiral limbus (SpL) in this $-/-$ mouse at 1-year of age. Staining with Toluidine blue. Original magnification: X1,000.

Figure 5.

In this 2-month-old $-/-$, the mesothelial cells of the basilar membrane (BM) are distended with lysosomal vacuoles (arrows). Staining with Toluidine blue. Original magnification: X1,000.

Figure 6.

a) Both the mesothelial and epithelial cells of Reissner's membrane contain lysosomal vacuoles (arrows) in this 2-month-old $-/-$. Staining with Toluidine blue. Original magnification: X1,000.; b) electron micrograph showing lysosomal storage vacuoles (arrows). Staining with uranyl acetate and lead citrate. Original magnification: X6,000.

Figure 7.

In this 6-month-old $-/-$, lysosomal vacuoles (arrowheads) were observed in the spiral ganglion cells (SG). Staining with Toluidine blue. Original magnification: X600.

Figure 8.

a) Lysosomal storage granules were never observed in the stria vascularis or hair cells. This 6-month $-/-$ mouse has a normal stria vascularis (S) and normal hair cells (arrows). Staining with Toluidine blue. Original magnification: X400; b) Electron micrograph of an outer hair cell (OHC) from a $-/-$ mouse at 6 months of age. TM indicates tectorial membrane. Staining with uranyl acetate and lead citrate. Original magnification: X5,000.

Figure 9.

a) By 12 months of age, organs of Corti (arrow) and neurofilaments (N) were missing in all $-/-$ mice. S indicates stria vascularis. b) This higher magnification of the neurofilament area shows some cells containing lysosomal vacuoles and a few remaining myelinated

neurofilaments. Staining with Toluidine blue. Original magnification: X400 (a) and X1,000 (b).