Syngeneic Bone Marrow Transplantation Reduces the Hearing Loss Associated With Murine Mucopolysaccharidosis Type VII

By Mark S. Sands, Lawrence C. Erway, Carole Vogler, William S. Sly, and Edward H. Birkenmeier

MPS VII mice are deficient in β-glucuronidase and share many clinical, biochemical, and pathologic characteristics with human mucopolysaccharidosis type VII (MPS VII). We have shown that syngeneic bone marrow transplantation (BMT) prolongs survival and reduces lysosomal storage in many organs of the MPS VII mouse. In this report, we quantify the hearing loss and determine the impact of syngeneic BMT on the development of deafness and the associated pathology in the MPS VII mouse. Eleven weeks after syngeneic BMT performed at birth, treated MPS VII mice had normal auditory-evoked brainstem responses (ABR), whereas untreated MPS VII mice had ABR thresholds 43 dB higher than normal. Treated MPS VII mice had β-glucuronidase-positive cells in the temporal bone and in the subepithelial connective tissue of the external auditory canal. There was less thickening of the tympanic membrane and middle ear mucosa and decreased distortion of the ossicles and the cochlear bone. Although transplanted MPS VII mice had increased ABR thresholds by 33 weeks of age, four of the six had thresholds 12 to 32 dB lower than untreated mutants. These data indicate that syngeneic BMT in newborn MPS VII mice prevents early hearing loss and, in some animals, results in long-term improved auditory function.

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MUCOPOLYSACCHARIDOSIS type VII (MPS VII, Sly Syndrome) is a lysosomal storage disease caused by the lack of β-glucuronidase activity and results in the accumulation of undergraded glycosaminoglycans in the lysosomes of many cell types. MPS VII is clinically and pathologically similar to many of the other mucopolysaccharidoses. Affected patients have progressive disease with bone and joint abnormalities, mental retardation, a shortened life span, and hearing loss. A mouse strain with a single base-pair deletion in the β-glucuronidase gene has a greater than 200-fold decrease in β-glucuronidase mRNA and no detectable enzyme activity. A canine model of MPS VII also exists that has approximately 1% normal levels of β-glucuronidase activity. Both the murine and canine model of MPS VII share many of the clinical, biochemical, and pathologic characteristics of the human disease and are excellent models to test the effectiveness of novel therapies such as bone marrow transplantation (BMT), gene therapy, and enzyme replacement.

Syngeneic BMT is currently being used to treat lysosomal storage diseases in humans. However, variable progression and severity of the disease at the time of transplantation, lack of HLA identical donors, and complications associated with BMT in humans can make evaluation of the therapeutic effect of this treatment difficult. The MPS VII mouse, on the other hand, has a uniform presentation of the disease on a defined genetic background. Syngeneic BMT performed in young adult mice with well-established disease is effective in reducing lysosomal storage material in many tissues including liver, spleen, mesangial cells of the kidney, corneal fibroblasts, and meninges. The life span of treated adult MPS VII mice increased to that of normal mice receiving the same form of therapy, but the skeletal system and neurons of the central nervous system (CNS) showed little or no improvement. BMT performed in newborn MPS VII mice, with minimal lysosomal storage, resulted in similar correction of the disease in most tissues and a dramatic improvement in the development of the skeletal system. After a low dose of conditioning radiation and BMT, the long bones grew to nearly normal lengths, there was less lysosomal storage in osteocytes and osteoblasts, and the synovium was cleared of lysosomal storage material. The pathology of the meninges was improved and β-glucuronidase-positive cells were detected in the brain. However, lysosomal storage was not consistently reduced in neurons and CNS function was not demonstrably improved because of the adverse effects of the conditioning radiation. Therefore, except for an increase in life span, clinical improvement could not be correlated with the reduction in storage apparent histologically.

Recently, a detailed morphologic analysis showed the presence of severe pathologic abnormalities in many structures of the ears of MPS VII mice. The abnormalities include sclerosis of the tympanic bulla and occlusion of the external auditory canal by cerumen. The tympanic membrane and middle ear (ME) mucosa are thickened with acute and chronic otitis media. Distended lysosomes occur in osteocytes in the tympanic bulla, ossicles, and cochlear bone. The joint capsules of the ossicles are thickened and synovial and periarticular fibroblasts are distended by cytoplasmic vacuoles. Malalignment and focal loss of stereocilia occurs as the disease progresses. These pathologic abnormalities could cause a conductive and sensorineural hearing loss similar to that observed in humans with mucopolysaccharidoses, including MPS VII.
Because the abnormal pathology in the ears and the hearing loss was prevented in MPS VII mice expressing a full-length human β-glucuronidase transgene, we determined the effect of other forms of therapy for treatment of the hearing deficits and ear histopathology. Here we show that MPS VII mice have a severe to profound hearing loss at 5 to 15 weeks of age and that published auditory-evoked brainstem response (ABR) thresholds are 42 dB higher than normal animals. This hearing defect was not evident in 11-week-old MPS VII mice treated with BMT as newborns. Histochemical and histopathologic studies after BMT in MPS VII mice showed β-glucuronidase-positive cells in the temporal bone adjacent to the ear and in the subepithelial connective tissue of the external auditory canal, and less severe histologic alterations in the ear compared with untreated MPS VII mice. These data indicate that BMT performed early in life dramatically improves the clinical and pathologic findings in the ears of mice with MPS VII. From these studies, we also conclude that ABR thresholds and ear pathology provide useful objective criteria for evaluating the effectiveness of somatic cell gene transfer and enzyme replacement therapies for MPS VII.

MATERIALS AND METHODS

**MPS VII mice and BMT.** Homozygous mutant (gus"+/gus"+) and phenotypically normal (+/+) mice were obtained from the B6.C-H-2"/ByJ-gus"/+ mutant strain maintained by E.H.B. at The Jackson Laboratory. Phenotypically normal (+/+) littermates include +/- and +/gus" mice. Heterozygous mice are completely normal by all criteria tested except for a 50% decrease in β-glucuronidase activity. Homozygous normal donors were obtained from a separate colony that is syngeneic except at the Gas-" locus. Animals for this study were either from a pedigree colony, maintained by strict brother-sister matings, or from a nonpedigreed colony in which the offspring were never more than one generation from the inbred colony. Five normal (+/-) and seven mutant (gus"+/gus") mice at 5 to 15 weeks of age were tested for ABRs to determine the extent of hearing loss in both sexes.

Normal mice were tested for ABR thresholds to determine the effect of age on hearing. Six mice from 9.5 to 11 weeks, five mice from 34 to 38 weeks, and four mice from 56 to 69 weeks of age were used for hearing. On the day of birth, seven +/+ and six gus"+/gus" mice were exposed to 200 rads delivered by a 60Co irradiator and received an intravenous injection of 100 μL bone marrow (BM) cells from the femurs of adult female +/+ mice. Five untreated, gus"+/gus" mice served as controls. The transplanted animals and untreated mutant controls were tested for ABR thresholds at 11 weeks of age and the transplanted animals were restested at 33 weeks of age. At 33 weeks of age, all of the untreated mutant controls had died.

**Hearing tests.** Mice were tested for hearing by means of the ABR as previously described. Each mouse was anesthetized with 0.2 to 0.5 mL of Avertin (tribromoethanol, 20 mg/mL) administered intraperitoneally. Needle electrodes were inserted subdermally on top of the head (active), below the left ear (reference), and on the dorsum (ground). Broadband clicks (1 to ±16 kHz) or pure-tone pips (8, 16, or 32 kHz) were delivered via headphones adapted with funnels loosely coupled to the pinnae of the mouse. The ABR threshold was determined for each stimulus in descending intensities (10 dB) and finally at ±5 dB. Each determined threshold exhibited at least two of the four or five peaks of the ABR waveform within 1 to 5 milliseconds after onset of the stimulus. ABR thresholds of 40 to 80 dB indicate a moderate to severe hearing loss, whereas thresholds above 80 dB indicate a profound hearing loss. Statistical significance of the hearing results was determined using Student’s t-test.

The 12 untreated mice initially tested (five mutant and seven phenotypically normal) were stimulated with broadband clicks generated from a Bio-logics averager (Traveler Express E, Mundelien, IL) and the remaining mice were tested with broadband clicks and with pure-tone pips generated with the Intelligent Hearing System averager. The results from the latter instrument were corrected as per calibrated acoustic output at the tip of the funnels. Broadband clicks stimulate most of the hair-cells over approximately the lower half of the frequency range of the mouse cochlea; thus, clicks evoke, from a normally functioning ear, a characteristic ABR waveform at the lowest thresholds. By contrast, pure-tone pips stimulate relatively few hair cells, which are attuned to each frequency. Age-related, sensorineural hearing loss generally progresses (raised thresholds) from highest to lowest frequencies, accompanied by loss of hair-cells from the base to apex of the cochlea, respectively. Conductive hearing loss, involving ME function, generally impedes hearing across all pip frequencies and broadband clicks.

**β-Glucuronidase activity.** The relative levels of donor BM engraftment were determined at 33 weeks of age by measuring β-glucuronidase activity in homogenates of liver from the transplanted mutant animals. In mice that were perfusion fixed, a section of liver was removed before fixation. β-Glucuronidase activity was measured using a fluorometric assay with 4-methylumbelliferyl-β-D-glucuronide (Sigma Chemical Co, St Louis, MO) as substrate. Protein determinations were performed by the Coomassie dye binding assay. β-Glucuronidase specific activity was calculated as nmol 4-methylumbelliferyl/μg protein and expressed as a percent of normal (+/+).

**Histochemistry.** Active β-glucuronidase was identified histochemically as previously described. Ear capsules were dissected from a 33-week-old treated MPS VII mouse, a treated normal mouse, and a 27-week-old untreated mutant. Ten-micron-thick cryosections of the ears were fixed for 30 minutes in chloral-formal-acetone fixative (0.3% chloralhydrate, 6% neutral buffered formalin, 70% acetone), then incubated 3 hours at 4°C in 0.25 mmol/L naphthol-AS-BI-β-D-glucuronide and hexazonized paraarsonil in 0.05 mol/L sodium acetate (pH 5.2) for 6 hours at 37°C. Stained sections were counterstained with 1% methyl green for 10 minutes.

**Pathology.** Ears from six BM-transplanted MPS VII mice, eight untreated MPS VII mice, and four untreated normal mice were examined by light microscopy. Included in this group were untreated MPS VII controls and mice that received 200 rads and syngeneic BMT at birth and were reported in previous studies but not tested for hearing. The histologic alterations were compared in age-matched mice. Heads were immersion fixed in 10% formalin or by transcardiac perfusion of anesthetized animals with ice-cold 2% glutaraldehyde and 4% paraformaldehyde in phosphate-buffered saline (pH 7.2) followed by immersion fixation. Ears were dissected from the skull and decalcified for several days in 10% formic, 40 mmol/L EDTA and embedded in paraffin or glycol methylmethacrylate. Eight-micron-thick transverse sections of the external, middle, and inner ear were stained with hematoxylin and eosin or toluidine blue.

RESULTS

**Hearing in untreated mice.** Untreated normal (+/+) and mutant (gus/+gus") mice from 5 to 15 weeks of age were tested for hearing using broadband clicks ranging from 1 to
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Normal mice have an average ABR threshold of 19 dB (±4.2) with a range of 15 to 25 dB. Untreated MPS VII mice have an average threshold of 61 dB (±8.0) with a range of 55 to 75 dB. The difference between the average ABR thresholds of normal and MPS VII mice is 42 dB.

ABR thresholds at broadband frequencies and 8, 16, and 32 kHz were compared in normal animals tested at 9.5 to 11, 34 to 38, and 56 to 69 weeks of age (Fig 2). As the mice age, there is a significant hearing loss at every frequency. Normal animals between 9.5 and 11 weeks of age have average ABR thresholds at broadband frequencies of 22.5 dB, which is similar to the normal mice described in Fig 1. At 34 to 38 weeks of age, the average ABR threshold increases significantly to 33 dB (P < .05) at the broadband frequencies. Corresponding increases in the average ABR threshold were also observed at the 8-, 16-, and 32-kHz frequencies.

Mice older than 1 year (56 to 69 weeks), have ABR thresholds significantly higher (P < .01) than mice at 34 to 38 weeks of age. There was no consistent difference between the auditory-evoked thresholds of male or female animals.

There is a minor difference (statistically significant at P < .1) in ABR thresholds between the untreated mutant mice described in Figs 1 and 3. This apparent difference is most likely because of the different instruments used to test the mice because a similar difference is observed between the age-matched untreated normals presented in Figs 1 and 2.

BMT corrects the hearing loss in MPS VII mice. Figure 3 shows the ABR thresholds of untreated and BMT-treated MPS VII mice compared with BMT-treated normal mice at 11 weeks of age. Compared with treated normal animals, untreated MPS VII mice of identical age had elevated ABR thresholds at every frequency. The increase in the average ABR threshold was 46, 46, 39, and 41 dB at the broadband frequency, 8, 16, and 32 kHz, respectively. The ABR thresholds of treated MPS VII mice did not differ significantly from those of treated normal animals. Compared with untreated MPS VII mice, the average ABR thresholds of the treated MPS VII mice were decreased by 43, 41, 40, and 36 dB at the broadband and 8-, 16-, and 32-kHz frequencies, respectively (Fig 3). The average difference in ABR thresholds at all frequencies between the treated normals and the untreated mutants was 43 dB. Similarly, the average difference between the treated mutants and untreated mutants was 40 dB.

The same mice treated with BMT were retested at 33 weeks of age. All of the treated mutants were still alive and clinically well, whereas all five untreated mutants had died. The ability of BMT to increase survival beyond the average 21-week lifespan of untreated MPS VII mice is consistent with our previous findings. Compared with 11 weeks of age, both the treated normal mice and treated mutant mice had elevated ABR thresholds at 33 weeks (Fig 4). Treated normals had an average increase of 13 dB at the various frequencies that was similar in magnitude to the age-related increases in ABR thresholds in untreated normal animals.
cells (red) in the temporal tissue of the external auditory canal (Fig 3). We estimate that the number of P-glucuronidase-containing cells is only 1% to 5% of normal. No P-glucuronidase activity was observed in a treated normal mouse of the same age; however, we estimate that the number of P-glucuronidase-containing cells is only 1% to 5% of normal. No P-glucuronidase activity was observed in an untreated MPS VII animal.

Biochemical and histochemical analysis. β-Glucuronidase activity was detectable in the liver of all treated MPS VII mice at 33 weeks of age. β-Glucuronidase activity in liver homogenates ranged from 0.6% to 1.7% normal, which approximated the levels previously reported in MPS VII mice receiving 200 rads of conditioning radiation and 1 x 10^6 syngeneic BM cells. A marked decrease in the pathologic alterations characteristic of murine MPS VII (Fig 6). In general, the tympanic membrane was thinner and there was less oitis in treated MPS VII mice compared with untreated MPS VII mice (Fig 6, A, B, and C). The ossicles (malleus [M] and incus [I]) still contained vacuolated cells but osteocyte alteration was less apparent, even in the oldest treated MPS VII animals, when compared with the same bones from untreated MPS VII mice (Fig 6, D, E, and F). However, the lysosomal storage material in chondrocytes appeared unchanged. ME mucosal thickening was present in a few of the transplanted MPS VII mice, but it was less severe and the large mucosal polyps present in untreated MPS VII mice were not observed. The cochlear bone from the treated MPS VII mouse shows marked improvement with reduction in the size of the peri-vascular spaces (Fig 6, G, H, and I). The external auditory canal (EC) of treated MPS VII mice still contained laminated cerumen in most cases.

Improved pathology of the ear after BMT. Histologically, the ears from the transplanted MPS VII mice showed a marked decrease in the pathologic alterations characteristic of murine MPS VII (Fig 6). In general, the tympanic membrane was thinner and there was less oitis in treated MPS VII mice compared with untreated MPS VII mice (Fig 6, A, B, and C). The ossicles (malleus [M] and incus [I]) still contained vacuolated cells but osteocyte alteration was less apparent, even in the oldest treated MPS VII animals, when compared with the same bones from untreated MPS VII mice (Fig 6, D, E, and F). However, the lysosomal storage material in chondrocytes appeared unchanged. ME mucosal thickening was present in a few of the transplanted MPS VII mice, but it was less severe and the large mucosal polyps present in untreated MPS VII mice were not observed. The cochlear bone from the treated MPS VII mouse shows marked improvement with reduction in the size of the peri-vascular spaces (Fig 6, G, H, and I). The external auditory canal (EC) of treated MPS VII mice still contained laminated cerumen in most cases.

DISCUSSION

The mucopolysaccharidoses are heritable disorders that affect many organ systems and cell types. Progressive conductive and sensorineural hearing loss are common clinical findings in the mucopolysaccharidoses, including MPS VII. Severe pathologic alterations have been observed in the ears of humans and mice with MPS VII. Here we show that β-glucuronidase-deficient mice have a severe to profound hearing deficit as early as 5 to 15 weeks of age.
The previously documented pathologic alterations are sufficient to explain a hearing loss with conductive and sensorineural components.15

Unlike untreated MPS VII mice, MPS VII mice that received BMT at birth have ABR thresholds that remain essentially the same as those of normal mice receiving the same treatment until at least 11 weeks of age. Although the dramatic improvement in hearing correlates with an improvement in histopathology of the ear, it is not clear which histologic finding or findings contribute significantly to the physiologic improvement. The presence of β-glucuronidase–positive cells in the marrow spaces around the ear and rare positive cells in the subepithelial connective tissue of the external auditory canal may directly contribute sufficient enzyme early in life to prevent or slow progression of the otic pathology. It was previously shown that only a small amount of enzyme activity (1% to 5% of normal levels) is required to reverse existing disease in the liver and spleen of adult mice.19,20 It was also shown that low doses of conditioning radiation followed by BMT at birth reduced many of the bone abnormalities in MPS VII mice, despite the lack of histochemically demonstrable β-glucuronidase activity in osteocytes.17 Although the amount of β-glucuronidase in the serum of transplanted mice is below the level of detection of our assay, it is possible that minute amounts of circulating enzyme may also contribute to the improved histopathology. Recombinant β-glucuronidase delivered intravenously to newborn mice results in dramatic improvement in many tissues including the bones and brain.22

The treated normal mice appeared to have a slight hearing deficit at 11 weeks of age compared with untreated normal mice. This deficit occurs before the age-related hearing loss and may be caused by the radiation-induced damage observed after BMT initiated at birth.13

Hearing loss occurs with age in both treated MPS VII and treated normal mice. The hearing loss in the treated normal mice is similar in magnitude to that observed with aging in untreated normal mice of the same strain. Although age-related hearing loss is normally observed in several mouse strains, histologic correlates for the defects have not been identified.23

The hearing loss by 33 weeks of age in some of the treated MPS VII animals was greater than that seen in age-matched normal controls. In fact, two of the treated MPS VII mice were deaf by this age. The otitis media and bony abnormalities characteristic of MPS VII were present, though less severe, than in untreated MPS VII animals. These abnormalities together with the external auditory canal cerumen may have contributed to the progressive hearing loss in some of the animals.

Although treated MPS VII mice had, on average, more hearing loss at 33 weeks of age than treated normal mice, the variation between individual animals was large. As mentioned above, two treated MPS VII mice were deaf by this age. The otitis media and bony abnormalities characteristic of MPS VII were present, though less severe, than in untreated MPS VII animals. These abnormalities together with the external auditory canal cerumen may have contributed to the progressive hearing loss in some of the animals.

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could have slowed the progression of the hearing loss and generated a more uniform response to BMT therapy.

Previous studies showed the efficacy of BMT as a means to reduce lysosomal storage in many tissues of mice with MPS VII. However, with the exception of the increase in longevity, it has been difficult to quantify the effect of BMT on any clinical or physiologic deficits associated with murine MPS VII. For example, we were unable to evaluate the effect of early BMT on the behavioral defects in the MPS VII mice because the conditioning radiation induced abnormalities in the normal mice receiving the same treatment. By contrast, the present study clearly shows that BMT at birth has a profound effect in preserving auditory function early in life.

BMT has been proposed as a means of therapy for human patients with MPS I, II, IV, and VII. Children with MPS I who received BMT at an early age have persistent levels of α-L-iduronidase, a decrease in intracranial hypertension, and slowed progression of their mental retardation. In these disorders for which BMT has been proposed, conductive and sensorineural hearing loss is an important clinical finding. A recent study of 12 children with MPS I who were treated with BMT showed that 8 of the children had improved auditory function to the point that they did not require amplification devices, whereas 4 showed no improvement. Our data on the MPS VII mice clearly show a slowed progression of the hearing deficits, β-glucuronidase–positive cells in and around the ear, and an improvement in otic histopathology. There is good correlation between the response seen in children with MPS I and mice with MPS VII which provides cause for optimism regarding the improvement in hearing by BMT for lysosomal storage diseases. Finally, regular assessment of ABR thresholds provides another parameter to evaluate the effectiveness of other novel therapies for these disorders such as gene therapy and enzyme replacement.

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