Corneal Opacity in Canine MPS I
Changes after Bone Marrow Transplantation

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Corneal opacification associated with glycosaminoglycan (GAG) deposition occurs in canine mucopolysaccharidosis I (MPS I), a deficiency of the lysosomal enzyme α-L-iduronidase. In affected dogs corneal lesions appear similar to those in children with the same disease. Transplantation of bone marrow from unaffected littermates was performed in 5 MPS I affected dogs at 5 months of age. In three recipients that became long-term survivors corneal clouding was largely alleviated compared to affected control dogs. In no case, however, did the corneas remain totally clear throughout the course of the study (594, 628 and 1425 days). Light and electron microscopic findings correlated with the clinical impression of partial improvement. Glycosaminoglycan analysis of corneal tissue from two transplant recipients, one normal littermate, and one MPS I-affected, untransplanted dog showed quantitative and qualitative changes in stored GAG following bone marrow transplantation. Invest Ophthalmol Vis Sci 30:1802-1807, 1989

The mucopolysaccharidosis (MPS) disorders are a group of hereditary diseases caused by defective catabolism of glycosaminoglycans (GAG), which occur in tissues as proteoglycans. An important clinical manifestation of MPS IH and IS (Hurler and Scheie syndromes), MPS IV (Morquio syndrome), and MPS VI (Maroteaux-Lamy syndrome) is the development of corneal opacification. In recent years, bone marrow transplantation (BMT) has been attempted as therapy for MPS with encouraging results. Metabolic correction, as assessed by leukocyte and tissue enzyme levels, urinary GAG excretion, normalization of hepatomegaly and splenomegaly and, in patients with MPS IH, a halt of mental deterioration, have been reported. Corneal clouding has cleared in patients with MPS IH, while no resolution occurred in a patient with MPS IV, although the patient’s vision did improve. In another child with MPS IV corneal clouding disappeared following BMT. Using the canine model of MPS I, we were able to study the biochemical and morphological changes associated with reduced corneal opacity following BMT.

Recently, we described a family of Plott hound dogs affected with MPS I (α-L-iduronidase deficiency). Clinically, the canine disease presents with intermediate severity, most closely resembling the human Hurler/Scheie phenotype of MPS I. All affected dogs develop corneal opacities by 1 year of age. The canine model was used to evaluate the effects of BMT in MPS I and we report here the changes observed in the cornea.

Materials and Methods

Five MPS I-affected dogs were given a bone marrow transplant from a tissue-matched littermate. All animal care and treatments were in compliance with the ARVO Resolution on the Use of Animals in Research. Three of the five recipients became long-term survivors. In this study, one normal bone marrow donor and one dog affected with MPS I were compared to two dogs with MPS I that received transplants and were subsequently killed 628 and 594 days post-BMT. Corneal tissue collected at the time of necropsy was frozen on dry ice and kept at −80°C until assayed. Methods for the isolation and quantitation of total corneal GAG have been described. Keratan sulfate (KS) was measured by the anthrone reaction and dermatan sulfate (DS) by a colorimetric procedure. Electrophoretic separation of GAG was performed on cellulose polyacetate (Sepharose III, Gelman Sciences, Ann Arbor, MI) in 0.1 M cupric acetate-

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acetic acid, pH 3.6, constant current 0.5 mA/cm for 2 hr. In each case, GAG equivalent to about 1 µg uronic acid was applied to the strips. Following electrophoresis the strips were immersed for 30 min without prior drying in 0.2% Alcian blue in 0.05 M magnesium chloride, 0.025 M sodium acetate and 50% v/v ethanol–water, and were destained for 45 min in a similar solution from which Alcian blue had been omitted. Corneal GAGs were identified by their mobility compared to GAG standards and by their susceptibility to the enzymes chondroitinase AC, chondroitinase ABC, testicular hyaluronidase and hyaluronidase from *Streptomyces hyalurolyticus*. Hyaluronidase from *S. hyalurolyticus* was assayed in 0.02 M sodium acetate–acetic acid buffer pH 6.0 in 0.15 M NaCl. Pronase (protease from *Streptomyces griseus*, 70,000 units/g) was purchased from Calbiochem-Behring Corp. (LaJolla, CA). The enzymes chondroitinase AC, chondroitinase ABC and hyaluronidase from *S. hyalurolyticus* were products of Seikagaku Kogyo Co. (Tokyo, Japan). Testicular hyaluronidase was purchased from Sigma Chemical Co. (St. Louis, MO). Cellulose polyacacetate strips (Sephracore III) 2.5 × 15.2 cm were the product of Gelman Sciences, Inc. (Ann Arbor, MI).

A portion of each dog's corneal tissue was also processed for routine histopathologic study and electron microscopy. Tissues were fixed for 1 hr in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. Following post-fixation in 1% osmium tetroxide for 1 hr and embedding in Epon 812, thin sections were stained with lead citrate–urananyl acetate, and examined on a Philips 201 electron microscope. Epon-embedded thick sections (1.5 µm) were stained with methylene blue–azure II for light microscopy.

**Results**

The overall clinical condition of the three long-term survivors was markedly improved and urinary GAG excretion was reduced to near normal levels by month 4 of the post-transplantation period. The most noticeable clinical improvement was amelioration or slowed progression of degenerative joint disease and lameness seen by 9–12 months of age in two untreated, littermate controls. Dogs receiving BMT grew larger than either affected, untreated dog, but remained smaller by 3–5 kg than the unaffected donors, which weighed 20–27 kg at maturity. At the time of BMT (5 months of age) corneal opacification was difficult to detect in any affected dog, but by 1 year of age both untreated, affected dogs had corneal cloudiness which increased in severity throughout the experimental period. Fundoscopic examination was impaired but revealed no lesions. Corneal clouding was markedly improved in two transplanted dogs (one necropsied on day 594 and one still alive) and moderately improved in the other (necropsied on day 628). Corneal neovascularization that responded to topical corticosteroid application was seen in each transplant recipient and may have been related to the effects of pre-BMT total body irradiation.

At the light microscopic level all lesions were in subepithelial layers of the cornea. There was marked vacuolation of mesenchymal cells (keratocytes) at all depths of the corneal stroma in the affected, untreated dog (Fig. 1). Cytoplasmic vacuoles, which often contained granular material (stored GAG), caused the cells to be much larger and distorted compared to those in tissue from the unaffected control dog. Both transplant recipients had a reduction in the...
Fig. 2. Cornea from an MPS I-affected dog that received a bone marrow transplant at 5 months of age from an unaffected littermate. Tissue collected 628 days post-transplantation. Mild vacuolation of some corneal cells persists, but is significantly reduced compared to the untransplanted dog. Methylene blue–azure II stain, x950.

number of corneal cells with cytoplasmic vacuolation and the degree of distension of involved cells (Fig. 2). The highest concentration of abnormal cells was located just below the corneal epithelium. Deeper regions of corneal stroma contained a higher percentage of mesenchymal cells with normal appearance. Vascular and perivascular cells in regions of neovascularization in the transplant recipients were normal morphologically. Alcian blue staining revealed large amounts of metachromatic GAG in the corneal stroma.

When examined with the electron microscope, expected differences between dogs were observed. The corneas of both transplanted dogs, as well as the control affected dog, contained mesenchymal cells with lysosomal distension, but the frequency and degree of lesions was much greater in the latter dog (Figs. 3, 4). In the untreated dog, lysosomal engorgement caused marked cellular enlargement and encroachment on the nuclei of keratocytes. Distended cells caused the intercellular collagenous fibrils to take on a wavy, in places disorganized, pattern. The corneal stroma of the untreated dog contained broad bands of homogeneous, slightly electron-dense material that caused separation of the bands of collagen fibrils (Fig. 3).

In both transplanted dogs there were individual

Fig. 3. Electron micrograph of corneal tissue from the same untreated dog with MPS I as in Figure 1 showing numerous lysosomes distended with stored GAG, causing cellular enlargement and distortion. Layers of homogeneous extracellular material between layers of collagen fibrils are present. Lead citrate–uranyl acetate stain, X5500.

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keratocytes with distended lysosomes, but overall there was a clear reduction in lysosome size and number. Less electron-dense material existed within vacuoles. The intercellular fibrils in transplant recipients were more linear and regular than in the affected control dog. The amount of homogeneous material between bands of collagen fibrils was also reduced (Fig. 4).

The GAG content and composition of the corneas is shown in Table 1. Consistent with the moderately severe corneal cloudiness, there was greater than 50% increase in total GAG content of the cornea from the affected, untreated dog compared to the normal control. The concentrations of individual GAG also changed considerably, both as a percentage of total and in actual amount. The concentration of DS, which is a minor constituent of normal corneal GAG, but whose metabolism is affected in MPS I, increased about 6 times. The amount of keratan sulfate and chondroitin–chondroitin sulfate (major normal corneal components, the metabolism of which presumably is not affected by α-L-iduronidase deficiency) decreased by 35 and 20%, respectively. Bone marrow transplantation resulted in only partial clearance of the stored DS from the corneas, and, while KS content reverted to near normal, chondroitin–chondroitin sulfate remained below normal levels.

Electrophoretic patterns of the corneal GAG prior to and after incubation with chondroitinases AC and ABC, and hyaluronidases, are shown in Figure 5. Lanes number 1, 7, 10 and 13 represent corneal GAG of a normal control dog. The lower part of chromatogram number 1 (intact GAG) was not susceptible to hyaluronidase from *S. hyalurolyticus* and, therefore, it is not HA. However, it is susceptible to chondroitinase AC (lane 7), testicular hyaluronidase (lane 10), and chondroitinase ABC (lane 13), and therefore, represents chondroitin. The upper part is largely unaffected by the various enzymes and represents mainly KS with smaller amounts of chondroitin sulfate.

<table>
<thead>
<tr>
<th></th>
<th>Total GAG (%) of LFDW</th>
<th>KS (mg/g wet weight)</th>
<th>C and CS (mg/g wet weight)</th>
<th>DS (mg/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>6.4</td>
<td>9.5 (67.8)†</td>
<td>3.5 (25.0)</td>
<td>1.1 (7.8)</td>
</tr>
<tr>
<td>MPS I, no BMT</td>
<td>10.1</td>
<td>6.2 (38.3)</td>
<td>2.9 (17.9)</td>
<td>6.9 (42.3)</td>
</tr>
<tr>
<td>MPS I post-BMT d.628</td>
<td>7.0</td>
<td>8.3 (58.9)</td>
<td>1.4 (9.9)</td>
<td>4.5 (31.9)</td>
</tr>
<tr>
<td>MPS I post-BMT d.594</td>
<td>6.6</td>
<td>7.6 (62.8)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* GAG = glycosaminoglycan; KS = keratan sulfate; C = chondroitin; CS = chondroitin sulfate; DS = dermatan sulfate.
† In parenthesis, % of total GAG.
†† LFDW = lipid-free dry weight.
NS = not studied.
Fig. 5. Cellulose acetate electrophoresis of GAG from canine cornea. Electrophoretograms represent GAG of cornea from (1) normal control dog, (2) MPS I affected control dog, (3) dog with MPS I, post-BMT day 594, (4) another dog with MPS I, post-BMT day 628, (5) GAG standards (HA, HS, DS, and C4-S), and (6) number 4 after incubation with chondroitinase AC. Lanes 7, 8 and 9 represent the same GAG as lanes 1, 2 and 3, but after incubation with chondroitinase AC. Lanes 10, 11 and 12 are the same as 1, 2 and 3, but after incubation with testicular hyaluronidase. Lanes 13, 14 and 15 are the same as 1, 2 and 3 after incubation with chondroitinase ABC. Lane 16 shows number 4 after incubation with testicular hyaluronidase. Lane 17 is a KS standard and 18 a C4-S standard. GAGs, about 1 µg as uronic acid, were applied on cellulose polyacetate strips (Sepharose III), and were subjected to electrophoretic separation in 0.1 M cupric acetate-acetic acid, pH 3.6, at a constant current of 0.5 mA/cm for 2 hr. Stained with Alcian blue.

fate and DS. Lanes 2, 8, 11 and 14 represent corneal GAG of the affected dog, and it is obvious that these consist mainly of DS. Lanes 3 and 4 represent intact corneal GAG of the two affected dogs on post-BMT days 594 and 628 and lanes 6, 9, 12, 15 and 16 the same GAG after incubation with various enzymes. It is evident that a considerable amount of the stored DS has not been cleared from the corneas and that the levels of KS and chondroitin are much lower than normal. Furthermore, the mobility of KS from the corneas of the affected dogs, both prior to and post-BMT, differs from that of the control, indicating that the degree of sulfation and/or the molecular weights are different from those of the normal control dog. The electrophoretic patterns of GAG from the same tissues, in a system using 0.1 M barium acetate, pH 8.0, constant current 1 mA/cm for 4.5 hr (in which DS and HA co-chromatograph), corroborated the findings in the cupric acetate system (results not shown).

Discussion

Corneal lesions at the light and electron microscopic levels in canine MPS I are similar to those reported in human MPS I.13,14 Stromal lesions are essentially identical, but the dogs lack the vacuolation of epithelial cells observed in people. Clinical benefits of BMT on corneal opacification in the MPS storage diseases have been variable. Encouraging results have been reported in children with MPS IH in whom clouding, when present, was obviously reduced in about 3–4 months, and nearly always completely cleared by 8 months post-BMT.3 Also, in feline arylsulfatase B deficiency (MPS VI), complete resolution of corneal clouding was reported.15 In contrast, human patients with MPS VI showed variable degrees of improvement in the appearance of their corneas following BMT.5,6 In the current study, despite the normalization of the excretion of urinary GAG, liver GAG and the substantial clearance of brain GAG, the resolution of corneal clouding was incomplete.

It is presumed that the underlying cause of corneal cloudiness in MPS is aberrant metabolism of corneal GAG. Corneal GAG, contained in proteoglycan molecules spaced regularly between collagen fibrils, may play a fundamental role in regulating the spacing of those fibrils.16 The presence of spaces containing homogeneous material between collagen fibrils greater than 2000 Å has been associated with increased light diffraction in patients with corneal clouding.17 Bands of extracellular GAG in MPS I-affected canine and human corneas likely cause gaps between fibrils that are greater than this critical distance. Our results may also shed some light on biochemical changes in the corneas of patients with MPS I.
The major corneal GAG of mammalian species are KS and chondroitin–chondroitin sulfate. In the tissue, they occur as proteoglycans, recently characterized as corneal keratan sulfate proteoglycan and corneal chondroitin–dermatan sulfate proteoglycan, respectively. The structure of the stroma in the adult chick cornea is characterized by tightly packed orthogonal layers of parallel collagen fibrils and by an abundance of KS. It is thought that the abundance of the latter in the stroma, is essential for corneal transparency. In rabbit corneal scars the amount of KS has been shown to be reduced.

In this study, we found that the major GAG of normal canine cornea were KS (about 65%) and chondroitin–chondroitin sulfate (about 25%). We also found a small amount of DS (less than 8%) and even less HA and HS. The 6-fold increase of DS content in the corneas of the affected dogs was more-or-less anticipated because of the deficiency of α-L-iduronidase. However, the decrease in KS content and the change in its electrophoretic mobility, which indicates a change in the degree of sulfation and/or molecular weight, was unexpected. If, as has been suggested, an abundance of KS (corneal keratan sulfate proteoglycan) organized with collagen in the stroma is essential for the structure and transparency of the tissue, these abnormalities could be responsible for the corneal opacity in MPS I. Alternatively, the corneal opacity may involve abnormalities in the chondroitin–dermatan sulfate proteoglycan. A plausible hypothesis regarding the abnormal metabolism of KS in corneal clouding is that the excessive, partially degraded DS inhibits biosynthesis of corneal keratan sulfate proteoglycan.

Considering the changes that occur in the corneal GAG in canine MPS I, and the partial correction after bone marrow transplantation, it is not surprising that incomplete resolution of the opacity was observed in both dogs.

Key words: cornea, biochemistry, pathology, mucopolysaccharidosis I, bone marrow transplantation

References