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Research article

A novel explanation of corneal clouding in a bone marrow transplant-treated patient with Hurler syndrome

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ABSTRACT

One common complication of mucopolysaccharidosis I-Hurler (MPS1-H) is corneal clouding, which occurs despite current treatments, including bone marrow transplantation. Human corneas were obtained from a 14 year old subject with MPS1-H and visual disability from progressive corneal clouding despite a prior bone marrow transplant at age 2. This was compared to a cornea from a 17 year old donated to our eye bank after his accidental death. The corneas were analyzed microscopically after staining with Alcian blue, antibodies to collagen I, IV, VI, and α-smooth muscle actin. Differences in levels of expression of the indicated molecules were assessed. Corneas from Hurler and control mice were examined similarly to determine potential mechanistic overlap. The MPS1-H subject cornea showed elevations in mucopolysaccharide deposition. The MPS1-H and Hurler mice corneas showed increased and disorganized expression of collagen I and IV relative to the control corneas. The MPS1-H corneas also showed increased and disordered expression of collagen VI. Positive expression of α-smooth muscle actin indicated myofibroblast conversion within the MPS1-H cornea in both the patient and mutant mouse material compared to normal human and control mouse cornea. Increased deposition of collagens and smooth muscle actin correlate with corneal clouding, providing a potential mechanism for corneal clouding despite bone marrow transplantation in MPS1-H patients. It might be possible to prevent or slow the onset of corneal clouding by treating the cornea with drugs known to prevent myofibroblast conversion.

1. Introduction

Mucopolysaccharidosis 1-Hurler (MPS1-H) is an autosomal recessive lysosomal storage disease caused by a mutation in the gene for α-L-iduronidase. This mutation results in an abnormal accumulation of glycosaminoglycans (GAGs), ultimately resulting in severe impairment of cell structure and function. MPS1-H manifests as a multi-system disorder, with a significantly reduced lifespan if untreated. A number of ocular findings have been described in these patients, including corneal clouding, optic nerve atrophy, glaucoma, and retinopathy. One of the most common complications is corneal clouding (Ashworth et al., 2006).

Treatments of MPS1-H patients with enzyme replacement therapy or bone marrow transplantation have significantly increased life expectancy as well as quality of life for these individuals (Tolar et al., 2008; Prasad and Kurtzberg, 2010). Short term post-transplantation studies, from 6 months to almost 3 years, demonstrated that bone marrow transplantation in these patients resulted in initial clearing of the corneal clouding, reduction in optic nerve edema, and improved retinal function (Summers et al.,

Abbreviations: MPS1-H, mucopolysaccharidosis I-Hurler; GAG, glycosaminoglycans; BCVA, best corrected visual acuity; PB, phosphate buffer; PBS, phosphate buffered saline.

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1989). Unfortunately, in time there can be the initiation and worsening of ocular abnormalities including corneal clouding, decreased visual function, and optic atrophy (Gullingsrud et al., 1998).

We obtained a cornea from a 14 year old patient undergoing a lamellar corneal transplant for corneal clouding 12 years after bone marrow transplantation for treatment of MPS1-H as well as an age-matched cornea. The cornea was examined for morphological changes and changes in collagen expression. Three types of collagen, each associated with a different family of collagen molecules, were analyzed. Collagen I is a fibril-forming collagen and is expressed in the cornea (Fitch et al., 1995). Collagen IV, the major collagen of basement membranes in the body, is known to be expressed in the corneal basement membrane and stroma (Kurpakus-Wheater et al., 1999), and is up-regulated in corneal scars after procedures such as radial keratotomy (Ljubimov et al., 1995). Collagen VI is a microfibrillar collagen and forms beaded filaments that play a role in tissue strength. It is thought to play an important role in the tensile strength and transparency of the corneal stroma (Cho et al., 1990). The up-regulation of collagen synthesis in the patient cornea suggested the potential for myofibroblast conversion, which was assessed by immunostaining for α-smooth muscle actin (Darby et al., 1990). All expression patterns were also assessed in the corneas of the mouse model for MPS1-H.

2. Methods

2.1. Tissue specimens

Collection of the corneal waste material was approved by all Institutional Review Boards (IRB). The use of Eye Bank tissue is exempt from IRB approval. All research complied with the tenets of the Declaration of Helsinki. Mouse tissues were collected from wild type control and from a mouse model of mucopolysaccharidosis I [MPS1-H] (Clarke et al., 1997). All mouse studies were approved by the Institutional Animal Care and Use Committee at the University of Minnesota and complied with the guidelines of the National Institutes of Health for the care and use of animals in research. Mice were obtained from a colony maintained by the Tolar laboratory, and housed by Research Animal Resources at the University of Minnesota. They were maintained in 12 h light/dark cycles and allowed to eat and drink *ad libitum*. Mice were sacrificed by CO_2 asphyxiation, followed by exsanguination. The corneas from 5 Hurler mice, with the same mutation as the MPS1-H subject, were examined and compared to corneas from 5 wild type mice.

2.2. Patient history

The cornea specimen from the 14 year old MPS1-H patient was obtained at the time of corneal transplantation surgery and frozen until sectioned. Procurement of the corneal tissue was obtained with patient permission, and complied with the requirements of the Health Insurance Portability and Accountability Act. The control cornea was obtained from the Minnesota Lions Eye Bank from a 17 year old donor who died of accidental trauma who had no history of eye disease, was frozen, and stored at −80 °C until sectioned.

The patient was diagnosed with MPS1-H with mild corneal clouding at age 2. The patient received a successful bone marrow transplantation from an unrelated donor (100% engraftment) at age 2 years. At age 10, a decline in visual acuity and worsening of corneal clouding was documented bilaterally. She continued to have progressive decline in visual function with increase in the corneal clouding. Her visual acuity dropped to best corrected visual acuity (BCVA) of 20/250 with a thick cornea (780 μm) despite relatively normal endothelial cells on specular microscopy. After a lamellar transplant in the right eye at age 14 years, her final BCVA was 20/30 with a corneal thickness of 570 μm. The cornea of the left eye was also cloudy, but was not operated on at this time. The cornea specimen from the MPS1-H patient was frozen and stored at −80 °C until sectioned.

2.3. Histological processing

The corneas were sectioned at 10 μm in a cryostat. A minimum of 5 sections from both the MPS1 and control corneas were stained with either hematoxylin and eosin (H and E) or Alcian blue to visualize the GAG accumulations. Sections were also immunostained for collagen I, IV, and VI as well as smooth muscle actin, which has been shown to identify myofibroblasts in tissue (Darby et al., 1990). For immunohistochemical localization in the human corneas, sections were rinsed in 0.1 M phosphate buffered saline (PBS), treated with 3% H_2O_2 in 0.1 M phosphate buffer (PB), rinsed in PBS, blocked in 10% normal serum, followed by overnight incubation at 4 °C in antibody against one of the following: collagen I (1:1000, abcam, Cambridge, MA); collagen IV (1:1000, abcam), collagen VI (1:1000, abcam), or α-smooth muscle actin (1:50, R&D Systems, Minneapolis, MN). For examination of corneas from a mouse model of mucopolysaccharidosis I as well as control mouse corneas, the globes were dissected, frozen, and sectioned at 12 μm. They were similarly immunostained for collagens and α-smooth muscle actin. For the mouse corneas, 1:2000 dilutions were used. After a PBS rinse, the sections were incubated using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA), and colorized by reaction with diaminobenzidine containing heavy metals. All immunostaining with a specific antibody was performed on the same day using the same reagents to ensure standardization.

2.4. Morphometric analysis

Densitometry for collagen and smooth muscle actin density was performed on 3 sections from the control and MPS1-H human corneas using the Bioquant (R and D Systems, Nashville, TN) morphometric analysis program. Multiple fields from each section were analyzed for each cornea and averaged. Data are presented as mean ± standard error of the mean.

3. Results

Microscopic examination of the MPS1-H cornea using standard H and E showed the stromal layer was thicker than similar regions of the normal age-matched control cornea, with a more basophilic and cellular appearance (Fig. 1). As mucopolysaccharidosis is a storage disease, we examined the corneas using Alcian blue, a staining method where the density of blue stain correlates with the amount of mucopolysaccharides in the tissues. The control cornea was relatively negative for Alcian blue staining (Fig. 2A), while the cornea from the MPS1-H subject showed a variable amount of reactivity depending on location in the cornea (Fig. 2B–D). Consistent with a prior report, these increased deposits of mucopolysaccharides were unevenly distributed (Constantopoulos et al., 1989). Similar results were seen in the corneas of the MPS1-H mouse corneas (not shown).

The MPS1-H patient cornea was immunostained for expression of three types of collagen, each associated with a different family of collagen molecules. Human corneas are largely type I and VI collagens (Robert et al., 2001), and so analysis of collagen staining was reflective of disorganization of the specific collagens examined. In the control human cornea, only low levels of disordered collagens I and IV were present in the stroma, with positive staining in a more orderly, linear pattern parallel to the corneal surface (Fig. 3A, C).