Type 1 Diabetes Mellitus' Influence on Corneal Epithelial Nerve Morphology and the Corneal Epithelium

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Abstract

Chronic, eye-threatening corneal pathology can arise from diabetic corneal neuropathy. Although the exact cause is unknown, it is thought that corneal disease is caused by a decrease in corneal sensitivity and loss of neurotrophic support. There is a lack of data on the connection between nerve damage to the corneal epithelium and nerve loss. In a streptozotocin-induced diabetic mouse model, we used three-dimensional imaging in vivo and in situ to investigate changes in nerve morphology and the corneal epithelium. Streptozotocin-treated mice displayed growth retardation and elevated serum glucose levels that were consistent with severe diabetes. After six weeks of disease, the length of the subbasal nerve plexus decreased. Corneal epithelial thinning and a decrease in basal epithelial cell density were linked to the subbasal nerve plexus's disappearance. On the other hand, the age of the animal was linked to the loss of the terminal epithelial nerves [1]. Importantly, this is the first rodent model of type 1 diabetes to exhibit corneal epithelial thinning and a decrease in basal epithelial cell density, both of which have previously been observed in diabetic corneal neuropathy patients. These discoveries demonstrate that in sort 1 diabetes, nerve fiber harm is clear in the sub basal nerve plexus before terminal epithelial nerve misfortune and that neurotrophic support from both the sub basal nerve plexus and terminal epithelial nerves is fundamental for the upkeep of corneal epithelial homeostasis.

Keywords: Corneal neuropathy; Corneal epithelium; Type 1 diabetes; Diabetes mellitus

Introduction

Diabetes mellitus (DM) is a serious metabolic condition that is becoming more common all over the world. At the ocular surface, loss of corneal innervation in DM is associated with reduced corneal sensitivity and altered tear secretion. Reduced aqueous tear production from decreased lacrimal gland innervation and an abnormal blink reflex contribute to the high incidence of dry eye that is frequently encountered clinically. Diabetic neuropathy is one of the most common complications of diabetes mellitus and increases in incidence with both age and duration of disease. Epithelial fragility, corneal erosions, and persistent epithelial defects are thought to be influenced by trophic support loss, which is necessary for epithelium homeostasis [2]. Changes in corneal thickness have also been reported. Restoration of corneal epithelial homeostasis and the associated corneal nerve plexus presents an important clinical challenge because many of these neurotrophic conditions are highly refractory to conventional therapies. In the earlier stages of disease, central corneal thickening occurs as a result of endothelial dysfunction and increased corneal edema. Later stages of disease, on the other hand, result in thinning of the corneal epithelium, which is associated with a more severe

In clinical research, the subbasal nerve plexus (SBNP) is increasingly examined using in vivo confocal microscopy (IVCM). The use of confocal microscopy in clinical studies prohibits examination of the terminal epithelial nerves (TENs) and raises many questions regarding whether the loss documented by the confocal examination represents one or more fibers, as well as the type of fibers affected. To address this gap, immunohistochemical studies using rodent models of diabetes have investigated the effects of hyperglycemia on the SBNP and TENs. Morphological alterations in the SBNP have been associated with both [3]. These studies have reported reductions in both the SBNP and the TENs using two-dimensional imaging techniques, with early loss of the TENs occurring before SBNP damage. However, due to the tortuous nature and intricate branching patterns of the TENs as they traverse the corneal epithelium, analysis of the TENs from two-dimensional data sets restricts the amount of data that can be extracted and necessitates more advanced three-dimensional (3D) modeling. Furthermore, neither central corneal epithelial thickness nor basal epithelial cell density (BECD), as seen in humans, have been found to be affected by disease or nerve loss in any of the rodent studies to date. In this study, we examined the effects of type 1 DM on the mouse corneal epithelium. Our primary objective was to assess the effects of type 1 diabetes on BECD and total corneal and sublayer thickness. Our secondary objective was to use 3D volumetric reconstruction of the SBNP and TENs in situ to evaluate the corresponding effects on neural architecture. Because of this, corneal nerve thickness and cellular changes in response to disease duration and animal age could be evaluated in a systematic manner [4].

Materials and Methods

Animals

This study made use of 31 male C57/BL6 mice that were 6 weeks old when they were purchased from the Mouse Breeding Core Facility at The University of Texas Southwestern Medical Center. In accordance with the approval of the University Institutional Animal Care and Use Committee, the high-dose streptozotocin (STZ) induction protocol from the Animal Models of Diabetic Complications Consortium was used to induce a type 1 diabetic state. Mice that had been fasting underwent a single i.p. injection of a citrate buffer with a pH of 4.5 and 150 mg/kg of STZ (Sigma, St. Louis, MO). Before being injected, the mice had to fast for four hours. Citrate buffer alone was injected into age-matched control mice. Mice were weighed and their serum glucose levels measured prior to treatment. A OneTouch Ultra Mini glucose monitor (LifeScan, Milpitas, CA) was used to collect blood from the tip of the tail vein for the purpose of measuring serum glucose. By day 3, diabetes was confirmed. After a minimum 5-hour fast, all serum glucose measurements were taken. A fasting serum glucose perusing more noteworthy than 300 mg/dL was viewed as diabetic. Normoglycemia persisted in all control mice. To prevent sudden hypoglycemia, mice received 10% sucrose water on the day of the injection. Mice were surveyed for body weight, serum glucose level, and corneal changes after one or the other 6 or 12 weeks of hyperglycemia, which compared to creature ages of 12 and 18 weeks, separately. Before enucleation, mice were euthanized under sedation by cervical disengagement. The Association for Research in Vision and Ophthalmology's guidelines for the use of animals in ophthalmic and vision research were followed in all cases [5].

Before scanning, mice were anesthetized with a single injection of 50 mg/kg ketamine, 5 mg/kg xylazine, and one drop of topical proparacaine 0.5% ophthalmic solution (Bausch & Lomb, Tampa, FL) using a modified Heidelberg Retina Tomograph Rostock Corneal Module (Heidelberg, Germany).Refresh Plus (Allergan, Irvine, CA) was used to maintain lubrication on the opposite eye. A silicon washer was positioned on the outer edge of the TomoCap (Heidelberg, Germany) to create a thin layer of space between the TomoCap tip and the surface epithelium in order to improve epithelial viewing. The washer's dimensions were as follows: 1.2 cm in diameter on the outside, 3 mm in diameter on the inside, and 600 millimeters thick (Specialty Silicone Products, Inc., Ballston Spa, NY). The corneas were scanned at a lens speed of 30 m per second in one-millimeter increments. On each cornea, a minimum of three scans were taken.

Immunofluorescence

Whole globes were removed and incubated for two hours at room temperature with gentle agitation in phosphate-buffered saline (PBS) containing 1.5 mg/mL Dispase (Gibco, Grand Island, NY). Corneas were extracted and fixed quickly in without rnase 4% paraformaldehyde (Electron Microscopy Sciences, Stronghold Washington, Dad) in PBS for 40 minutes at room temperature. After being washed with PBS, corneal tissue was permeabilized and blocked for two hours at room temperature using PBS containing 1% bovine serum albumin and 0.2 percent Triton X-100 (Sigma, St. Louis, MO). After that, 3.3 g/mL of rabbit polyclonal antibody against neuronal class III-tubulin (MRB-435P; Covance, Emeryville, CA) overnight at 4°C in PBS containing 1% bovine serum albumin and 0.2 percent Triton X-100. After being washed, corneas were incubated with a secondary antibody conjugated to fluorescein isothiocyanate, 6.7 g/mL (Alexa488; Cell Signaling, Danvers, MA) for 2.5 hours at room temperature with 0.5% bovine serum albumin and 0.1% Triton X-100 in PBS. Iodide of propylene (5 g/mL; For the final hour, counterstaining the nuclei of epithelial cells (Invitrogen, Grand Island, NY) was added. Using a 75% vol/vol solution of glycerol–PBS, corneas were mounted on glass-bottom tissue culture dishes (MatTek, Ashland, MA) and imaged with a Leica SP2 laser scanning confocal microscope (Leica Microsystems, Heidelberg, Germany). To assess the SBNP's overall distribution, a single 20 image magnification was taken of each cornea. Three representative images were taken with a 63 water objective at a 2.0 image zoom for subsequent image processing and analysis.

IVCM

IVCM was used to evaluate in vivo changes in the cornea. Estimations of complete corneal, epithelial, and stromal thickness values were resolved utilizing our in-house confocal microscopy through centering program. For in situ morphologic estimations, informational indexes were gotten utilizing a laser checking confocal magnifying lens and used to produce 3D picture stacks. MetaMorph software version 7.7.0.0 was used to manually count propidium-iodide-stained nuclei within the basal epithelial cell layer in order to measure BECD from the 63 image stacks. Imaris software version 7.3.1 was used to measure the total length of the SBNP and TEN nerve fibers [6]. Surface renderings were created within Imaris to three-dimensionally model the corneal nerves and epithelium. Then, all TENs and subbasal nerve fibers were traced using the Filament Tracer function. This made it possible to produce a model that was the most accurate representation of the nerve plexus by combining operator decision making with automated analysis. Separately, the SBNP and TENs were examined. The software program automatically measured the total length of the nerve fibers from the SBNP and TENs in the resulting 3D models after the volume renderings were finished. A masked observer carried out every measurement.

Results

Impacts of stz-actuated type 1 diabetes on body weight and serum glucose levels

All mice infused with STZ neglected to put on weight contrasted and the vehicle-infused controls. When compared to diabetic mice, the control animals' body weight increased by 25.8% at 12 weeks. Similarly, control mice's body weight increased by 29.2% at 18 weeks, whereas diabetic animals' weight did not change from baseline. All STZ-treated mice outgrew age-matched controls in a way that was consistent with a failure to thrive. At the outset, there was no statistically significant difference in the body weight of STZ-injected mice or vehicle-treated controls. In contrast to body weight, the STZ-treated mice showed a significant rise in serum glucose levels. At 12 weeks, the serum glucose level of the STZ-treated mice was 194.4 percent higher than that of the controls. At 18 weeks, the serum glucose level in STZ-treated mice was also 215 percent higher than in controls. At baseline, there was no statistically significant difference between the two groups.

Effects of type 1 diabetes on the corneal epithelium

IVCM was used to evaluate the effects of type 1 diabetes on the surface epithelium and cornea of a full-thickness mouse. Figure 2 and Supplemental Video S1 present representative images. The total thickness of the cornea decreased by 7.2% after 12 weeks of diabetes caused by STZ. The sublayer corneal thickness revealed that this effect was mediated by a decrease in stromal thickness (10.2% thinner in the STZ-treated group than in the controls), while the thickness of the corneal epithelium was only reduced by 1.6%. At 18 weeks of illness, the complete corneal thickness was diminished fundamentally by 10.2% in STZ-treated mice contrasted and controls (P = 0.011). This was accompanied by significant thinning of the corneal epithelium (a decrease of 12.2% when compared to controls; P = 0.026). Stromal thickness also decreased by 9.3% in this group, though this decrease was not significant.

Effects of type 1 diabetes on the subbasal plexus and TENs

The three distinct nerve morphologies that were observed in the 3D volumetric images are as follows: simple, resonant, and intricate. Figure 4 depicts representative images.. Ramifying nerves, like simple nerves, branched out from the SBNP and ran anteriorly toward the surface of the cornea. Ramifying nerves, on the other hand, continued to run parallel to the SBNP through superficial cell layers after branching out in either the wing or squamous epithelial cell layers [7]. The length of these parallel fibers varied greatly and frequently extended over distances greater than three to five epithelial cells. Complex TENs, in contrast to simple and ramifying nerves, showed evidence of anastomoses between more apically localized nerve fibers and a multibranching pattern among wing and surface epithelial cells.

In comparison to the vehicle-treated control, low-magnification views of the SBNP revealed visible nerve fiber degradation in the diabetic cornea, including focal loss within the whorl-like vortex. The overall length of the TENs that were observed branching from the remaining subbasal nerve fibers appeared to remain relatively unchanged within the scattered areas of SBNP damage. Figure 5, C–F, as well as Supplemental Videos S2, S3, S4, and S5, provide representative 3D renderings and segmentations of the SBNP and TENs that are produced in corneas from diabetic patients and healthy individuals. Just a little extent of subbasal nerve filaments showed proof of TEN stretching in the typical cornea. In contrast, there were fewer nonbranching fibers observed in the diabetic cornea [8].

Discussion

There were two critical discoveries in this review. In the severe diabetic state, the central corneal thickness first decreased. Both the stroma and the epithelium revealed the decrease in corneal thickness. Previously, Rosenberg et al. used tandem scanning confocal microscopy to identify epithelial thinning in patients with significant corneal neuropathy and reported clinical changes in corneal thickness in diabetes patients. However, corneal edema was cited as the cause of an increase in stromal thickness. We found that in the diabetic state, stromal thickness decreased at both disease time points and was associated with significant growth retardation [9]. This finding suggests that stromal development may be affected or disrupted by the widespread effects of hyperglycemia. It has been reported that diabetes alters stromal biomechanical properties, but this has not been investigated. These include abnormalities in collagen organization and an increase in corneal hysteresis, which has been hypothesized to be caused by an increase in the accumulation of advanced glycation end products. An important area of future research is the impact that chronic hyperglycemic stress has on keratocyte function and stromal remodelling [10].

In studies of the central corneal and epithelial thickness in the C57/BL6 mouse cornea, it has been reported that, in the absence of disease, murine corneal thickness values increase during the first four to eight weeks of life before leveling off and remaining relatively unchanged throughout adulthood31. Henriksson et al. measured the epithelium and stroma at 40.59 and 90.88 m, respectively, using conventional light microscopy on glutaraldehyde-fixed tissue in situ. They found a mean central corneal thickness of 137.02 m. Using two-photon imaging, Zhang et al.33 evaluated corneal thickness in vivo in C57/BL6 mice, obtaining central values of 116.6, 39.9, and 76.7 m for the total, epithelial, and stromal thicknesses, respectively. In this study, the researchers reported a central corneal thickness value of 106 m for adult mice in vivo compared to 141 m for fixed tissue in situ. Spectral domain optical coherence tomography has also been used to measure corneal thickness in vivo and in situ. Sublayer thickness values were not reported because optical coherence tomography had limitations [11]. At approximately 12 and 18 weeks of age, respectively, total corneal thickness values in control mice were 105.9 13.6 m and 108.7 4.8 m using IVCM. The utilization of IVCM for the purpose of determining corneal thickness in this study is supported by these findings, which are consistent with the previous reports on in vivo optical coherence tomography.

The second major finding of this study was that hyperglycemia was associated with a shorter total length of the SBNP, whereas normal maturation caused a shorter number of TENs. This response was greatest at 18 weeks and is consistent with duration of disease as a risk factor for diabetic neuropathy [12].1 Multiple mechanisms have been proposed to play a role in the onset and progression of diabetic neuropathy. This response to prolonged hyperglycemia is consistent with previous rodent and human clinical models. Alterations in metabolic and signaling pathways, vascular insufficiency, and inflammation and oxidative stress caused by hyperglycemia are examples of these [13]. It has been demonstrated that the length of the SBNP's nerve fibers decreases when the posterior ciliary artery's acetylcholine-mediated vascular relaxation decreases. As a result, the SBNP may be deteriorating as a result of systemic diabetes-related factors in addition to locally derived mediators. This is the first rodent model of type 1 diabetes that demonstrates corneal epithelial thinning and changes in BECD associated with diabetic corneal neuropathy that are comparable to changes reported in humans [14]. This has been supported by clinical studies that showed that an enhancement in SBNP morphology is associated with reductions in systemic risk factors associated with diabetes as well as successful pancreas transplantation.

Conclusions

These findings suggest that neurotrophic support from both SBNP and TENs is necessary for the maintenance of corneal epithelial homeostasis and that the pathophysiological effects of hyperglycemia affect SBNP prior to TEN loss. It is essential to clarify the pathophysiological mechanisms underlying these changes because changes in the corneal nerve architecture and corneal sensitivity are increasingly being used as outcome measures in studies evaluating the severity of diabetes, comorbidity, and therapeutic modalities. To confirm and extend these findings, additional studies utilizing human donor corneal tissue are needed to systematically investigate these changes.

Acknowledgement

None

Conflict of Interest

None

References

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