Original Research *Highlight article*

Blockade of OGFr delays the onset and reduces the severity of diabetic ocular surface complications

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Impact statement

This research demonstrated for the first time that blockade of the OGF–OGFr regulatory pathway with the potent opioid receptor antagonist, naltrexone (NTX), alters the temporal onset and magnitude of ocular surface complications associated with type 1 diabetes. Daily systemic NTX treatment delayed the onset of dry eye and decreased the magnitude of abnormal ocular surface sensitivity. Topical NTX therapy, administered twice daily, prevented the onset of dry eye and aberrant corneal surface sensitivity such that no abnormalities appeared during the entire eight-week study. Both systemic and topical NTX enhanced corneal epithelial wound healing rates in diabetic rats, and reduced tissue and serum levels of OGF, an inhibitory growth factor. These data support the hypothesis that the OGF–OGFr axis is dysregulated in diabetes, and demonstrate that blockade by NTX protects against diabetic ocular surface complications.

Abstract

The opioid growth factor (OGF)–OGF receptor (OGFr) pathway is present in the ocular surface and functions to maintain homeostasis of the epithelium. The OGF–OGFr pathway has been reported to be dysregulated in diabetic individuals and animal models, and is reflected in elevations of the inhibitory growth factor, OGF, chemically termed [Met^5]-enkephalin. Recently, our laboratory reported elevated levels of OGF and OGFr in the serum and corneal epithelium of type 1 diabetic rats, suggesting that dysregulation of the OGF–OGFr axis may lead to dry eye, abnormal corneal surface sensitivity, and delayed re-epithelialization. Blockade of OGF–OGFr pathway using naltrexone, a potent opioid receptor antagonist, reverses dry eye symptoms and restores corneal surface sensitivity in diabetic rats when used as a therapy. Based on the evidence that both OGF and OGFr are elevated in type 1 diabetic rats, this study examined whether systemic or topical naltrexone treatment initiated at the time of induction of hyperglycemia could protect against the development of diabetic ocular surface complications. Diabetic male Sprague-Dawley rats treated systemically or topically with naltrexone had a delayed onset of dry eye and altered corneal surface sensitivity, and an improved healing rate for corneal wounds, that were comparable to non-diabetic rats. Serum levels of OGF were normal for rats receiving systemic naltrexone, and OGF tissue levels were normal for type 1 diabetic rats receiving twice daily naltrexone drops. OGFr levels remained elevated. These data support the role of the OGF–OGFr axis in regulation of ocular surface complications, and suggest that naltrexone therapy may be beneficial for pre-diabetic and early diabetic individuals.

Keywords: Hyperglycemia, dry eye, keratopathy, serum OGF, OGFr, corneal re-epithelialization

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Introduction

Diabetes is a major healthcare concern in the United States, accounting for over $327 billion in direct medical costs and lost productivity.[1] Diabetes disproportionately affects Americans over the age of 65 (>26%), and those of Hispanic (~12%) and non-Hispanic black (~11.7%) ethnicity.[2–4] Complications affect nearly every individual with diabetes at some time in the course of the disease. With more than 124 million people in the United States population diagnosed with pre-diabetes or diabetes,[1–3] this highlights the unmet medical need to examine the onset and magnitude of complications, and begin to understand their underlying pathophysiology. Specifically, nearly 50% of all individuals diagnosed with type 1 diabetes (T1D) will experience one or more ocular complications.[4,5] Clinical and pre-clinical studies have reported that diabetes is associated with the onset of dry eye, abnormal corneal surface sensitivity, chronic non-healing wounds, and retinopathy,[5–9] but few reports have identified a specific causal relationship.

Our laboratory has investigated the role of the opioid growth factor (OGF)–OGF receptor (OGFr) pathway in maintaining corneal surface homeostasis.[10–12] Studies on this growth regulatory pathway have shown that diabetic

rats, rabbits, and mice have decreased corneal epithelial turnover, delayed corneal abrasion repair, reduced tear production, and decreased corneal sensitivity.[13–18] Treatment of diabetic mice and rats with naltrexone (NTX), a potent opioid antagonist, normalized epithelialization[13–16,18,19] and reversed dry eye.[17–19] These results imply that one or more of these factors related to the OGF–OGFr regulatory axis are aberrant in diabetes. Several reports have suggested that humans and animals with diabetes have elevated serum enkephalin levels.[20–22] OGF is chemically termed [Met^5]-enkephalin, and functions as an inhibitory growth factor. Elevated serum levels of OGF and its selective receptor, OGFr, were reported in a study of T1D male rats as early as two weeks after the induction of hyperglycemia.[22] Corneal epithelial tissue had elevated levels of both OGF and OGFr, suggesting that there is a dysregulation of this pathway that delays re-epithelialization following corneal abrasion. We hypothesize that the elevated tissue levels of the inhibitory peptide lead to abnormal tear production and corneal surface sensitivity, as well as delayed and/or incomplete repair. The elevated serum levels also may have systemic impact on a variety of physiological pathways that have yet to be identified or studied.

In the present study, we investigated whether blockade of the OGF–OGFr axis with NTX could prevent the onset and magnitude of the consequences of dysregulation that have been observed in the pathway in T1D. The hypothesis of the research is that NTX blocks the interaction of the inhibitory peptide OGF with OGFr and thus prevents, prolongs, and/or decreases ocular surface complications associated with diabetes. Therefore, different paradigms of systemic NTX injections and topical NTX application were investigated to determine whether the course and magnitude of ocular surface complications in T1D could be prevented or delayed. In humans, delaying the onset of dry eye or abnormal surface sensitivity would be advantageous as would decreasing the magnitude of other diabetic-related ocular surface complications such as delayed corneal epithelial healing.

Materials and methods

Type 1 diabetes rat model

The methodology for induction of hyperglycemia followed published procedures.[14,19,22] Briefly, six-week-old fasted male Sprague-Dawley rats (Charles River) were injected intraperitoneally (i.p.) with 55 mg/kg streptozotocin (STZ; Sigma-Aldrich) on two consecutive days.[22] Blood glucose levels were measured using a TruTrack® glucometer, and tail blood readings of >300 mg/dL indicated that the rat was hyperglycemic (T1D). A subset of STZ-injected rats received insulin implants (LinShin, Canada) within 24 h of detecting hyperglycemia (= T1D-INS). Insulin was released within 1 h of implantation at a rate of 2 U/h. Another group of rats received i.p. injections of sodium citrate and were considered "Normal". All animal protocols were approved by the Institutional Animal Care and Use Committee of the Penn State College of Medicine and complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Treatment paradigms testing blockade of OGFr with naltrexone

Three experimental paradigms, designed to assess treatment of T1D, were conducted sequentially. In the first experiment, systemic treatment of NTX was investigated in male T1D rats by i.p injection of 30 mg/kg NTX ($T1D_{Sys\text{-}NTX}$) daily for eight weeks beginning with confirmation of hyperglycemia. In the second and third experiments, NTX was administered topically to the corneal surface either once daily ($T1D_{TopNTX\text{-}1drop}$) or twice daily ($T1D_{TopNTX\text{-}2drop}$). Each drop contained 5×10^{-5} M NTX dissolved in saline (pH 7.0) and was administered between 0800 and 0900 or 1500 and 1600 daily for eight weeks. A final group of T1D rats received only saline by systemic injection or eye drop. Another set of controls were Normal, non-diabetic animals receiving saline either systemically or topically. No differences were noted in parameters of saline-treated T1D or saline-treated Normals rats, and thus data were combined for analyses. In some experiments, T1D animals receiving insulin implants were included for comparison (T1D-INS). Body weights and blood glucose levels were monitored periodically throughout the eight-week period.[22] Multiple cohorts of animals were required for systemic and topical NTX studies, and at least 8–15 rats were included in each treatment group. Any rat that did not convert to hyperglycemia within four days following STZ injection was removed from the study.

Ocular surface complications

Following protocols established previously,[19,22] tear production and corneal surface sensitivity were assessed weekly for 8 weeks beginning 14 days after confirmed hyperglycemia. Both measurements were performed on unanesthetized rats. Briefly, *tear production* was determined using the Schirmer 1 tear test that measured the wetting length of Schirmer paper strips placed in the lower lid cul-de-sac. *Corneal surface sensitivity* was assessed by recording a positive blink response using the Cochet-Bonnet aesthesiometer (Boca Raton, FL). After six weeks of NTX treatment, a subset of male rats in the Normal, T1D, $T1D_{sys\text{-}NTX,}$ $T1D_{TopNTX\text{-}1drop}$, and $T1D_{TopNTX\text{-}2drop}$ groups were subjected to surgical corneal abrasions.[15] *Corneal epithelial wound healing* was assessed as the percentage of residual defect at 24, 32, 48, and 56 h following surgery. Corneal abrasions were surgically created between 0800 and 0900 h to prevent disparities from diurnal rhythm. The percent residual wound for each rat was determined photographically by tracing the area of fluorescein-stained cornea and calculating the percentage change from the original baseline corneal wound (0 h). No rat was photographed more frequently than every 24 h in order to prevent disruption of the epithelial healing process. Treatment regiments of topical or systemic NTX or saline were maintained throughout the corneal wound healing process.

Serum and tissue levels of OGF and OGFr

At four and eight weeks of hyperglycemia, rats from each treatment group were humanely euthanized by injection of sodium pentobarbital (Euthasol) injection (>100 mg/kg; >0.2 mL/100 g body weight). At eight weeks, eyes were proptosed in three rats per group, rapidly frozen in isopentane, and stored at -80°C for morphological assessment. Blood was collected at four and eight weeks and isolated serum was stored at -80°C. OGF and OGFr protein expression levels were determined by immunohistochemistry. Corneal epithelial histologic sections were stained with validated antibodies using published procedures[22]; sections stained with secondary antibody only were negative controls. Positive controls included ocular tissue from earlier investigations. Images were calibrated using optical density step tablet software provided by ImageJ, such that densitometric analyses removed the mean gray background values and converted data from pixels to optical density units. Corneal tissue (at least 10 sections/treatment group) was examined with confocal microscopy (Keyence Microscopy) and the intensity of fluorescent staining measured using Image J software. Densitometric analyses were performed on photographs captured at similar settings and magnification.

OGF and OGFr serum levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, CA). OGF (MBS756126), OGFr (MBS109224), as well as neprilysin CD10 (Cluster of differentiation 10), a peptidase for enkephalin (MBS764927) were assayed with kits that were specific for rat. The manufacturer's guidelines indicated high sensitivity for the indicated peptide/protein. Two or more ELISA assays were conducted with duplicate samples from four to seven different rats at each time point; samples from other assays, as well as previous experiments, were included as positive controls.

Serum levels of naltrexone

To investigate the pharmacokinetic tissue dispersion of NTX, serum samples from rats systemically treatment with NTX, or those treated twice daily each time with a drop of 5×10^{-5} M NTX, for eight weeks were assayed using a Naltrexone ELISA kit (Neogen Lot #NLF-0006; Lexington, KY). For comparison, a positive control included normal serum spiked with 1 μg NTX, and a negative control of unaltered rat whole serum.

Data analyses

Rigor and reproducibility of the study were ensured by conducting multiple consecutive experiments with smaller cohorts of rats and using replicate samples from multiple animals, as well as samples from previous studies as positive controls in some assays. Antibodies and assay kits were validated by commercial vendors. Inasmuch as possible, new data (e.g., body weights and glucose measurements) were confirmed with published values. All parametric data were analyzed with GraphPad Prism 8.0 software using one-way or two-way (condition, time point) analysis of variance (ANOVA) to determine significant interaction. If significant interactions were noted (greater than 95% confidence levels), further analyses and comparisons with Newman–Keuls tests were completed.

Results

Clinical characteristics

More than 97% of all rats injected with STZ became hyperglycemic (had blood glucose levels that were >300 mg/dL) within 72 h; any STZ-injected animal that did not become hyperglycemic was removed from the study. No animal died of experimental procedures. Two-factor ANOVA indicated that there was no interaction between time and treatment, thus one-way ANOVAs were conducted. Body weights were recorded bi-weekly and data at four and eight weeks are presented in Figure 1(a). The patterns of body weights were comparable at both time points. T1D-INS rats weighed comparable to Normals, whereas all other T1D rats had substantially lower body weights relative to Normals. Blood glucose levels (Figure 1(b)) were significantly elevated at four and eight weeks in all T1D and NTX-treated T1D groups in comparison to both T1D-INS and Normal values.

Protection or delay in the temporal onset and magnitude of corneal surface complications by NTX treatment

Decreased tear production and reduced corneal surface sensitivity are hallmark complications that appear coincident with the development of diabetes (Figure 2). In an earlier report, dry eye was recorded at four weeks post STZ injections and was consistently evident for eight-weeks.[22] Likewise, altered surface sensitivity requiring greater pressure to elicit a blink response, also was noted beginning one month after STZ injection, and remained constant throughout the eight-week study for T1D and T1D-INS rats.[22] In the present study, two-factor ANOVA revealed significant interactions between condition and time ($P < 0.0001$) for tear fluid production and surface sensitivity, with significant main effects indicated at each time point. Relative to Normal rats, the corneal sensitivity in T1D and T1D-INS groups was decreased beginning on week 4 through week 8, requiring increased force to elicit a response (Figure 2(a)). Daily systemic NTX treatment significantly delayed the onset of aberrant corneal surface sensitivity by three weeks and reduced the magnitude of decreased sensitivity at four, five, six, and seven weeks (Figure 2(a)). At week 8, the rats receiving NTX systemically required the same force to elicit a response as T1D animals. Animals receiving one drop of NTX daily had normal corneal sensitivity until week 6 when pressure values differed from Normal. However, these rats displayed a significantly reduced magnitude of defects relative to T1D rats on weeks 6–8. Rats receiving two drops of NTX had corneal surface sensitivity measures comparable to Normal for the entire eight-week period, suggesting a "protective" effect in this parameter.

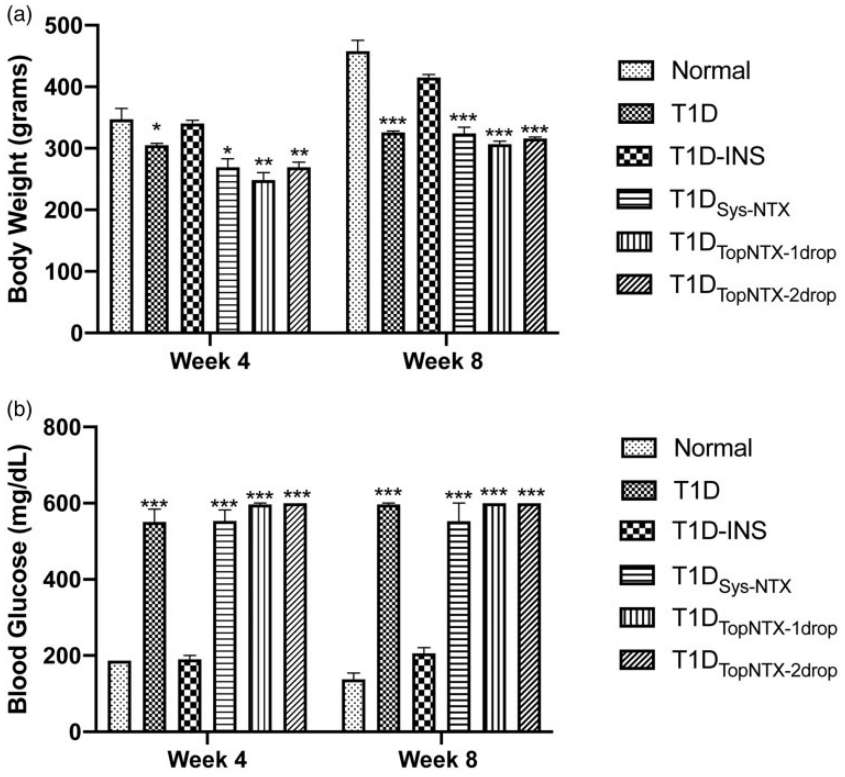

Figure 1. Body weight (grams) (a) and blood glucose levels (mg/dL) (b) for male Sprague-Dawley rats at four and eight weeks following STZ injections. Animals ($n = 6$–8 per group) were randomly divided into groups at the time of confirmed hyperglycemia and received either saline (T1D), daily intraperitoneal (i.p.) injections of 30 mg/kg NTX (T1D$_{Sys-NTX}$), or topical administration of one drop (5×10^{-5} M NTX) once daily (T1D$_{TopNTX-1\ drop}$) or twice daily (T1D$_{TopNTX-2drop}$). For comparison, a cohort of T1D rats received insulin implants (T1D-INS) but no further treatment, and some rats received buffer instead of STZ and were considered Normal. Data were analyzed with one-factor ANOVA and Newman–Keuls tests as there were no significant interactions between Condition and Time. Values represent means ± S.E.M. Significantly different from Normal values at $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

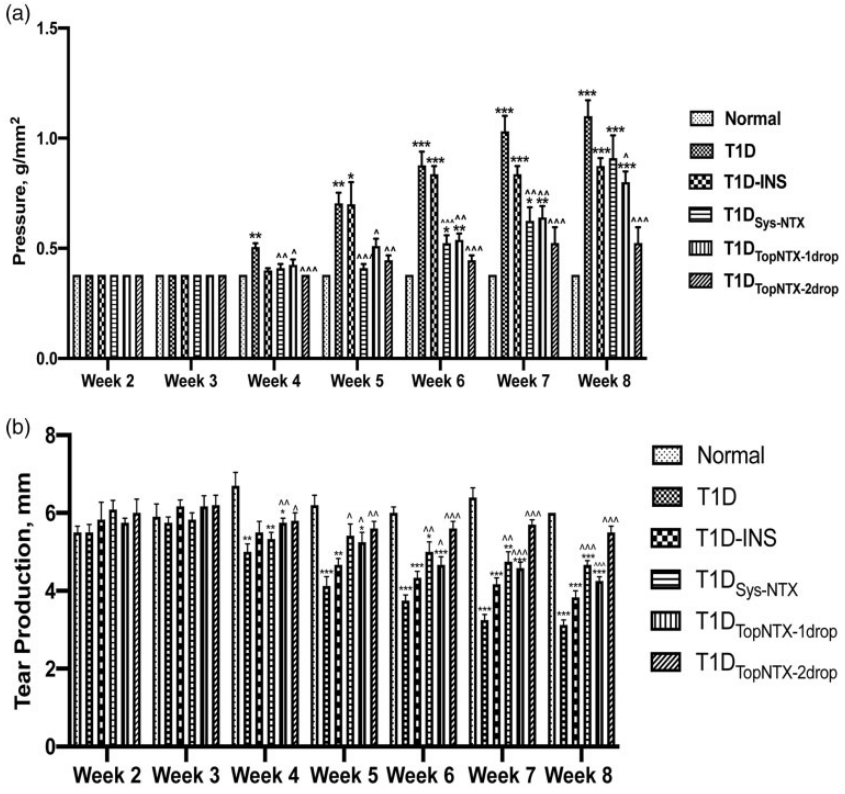

Figure 2. Corneal surface sensitivity (a) and tear fluid production (b) measured weekly beginning two weeks after injection of STZ for rats in each group as described in the legend for Figure 1. Sensitivity was measured with a Cochet-Bonnet aesthesiometer. Three readings of pressure (g/mm^2) were averaged for each rat at every time point. Tear production (mm of wetness) was measured by the Schirmer 1 tear test. Values represent means ± S.E.M. for male rats; $n = 6$–8 animals per group at each time point. Two-way ANOVA revealed significant interactions ($P < 0.0001$) for both parameters justifying further analysis. Significantly different from Normals at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and significantly different from the T1D values at $P < 0.05$ (^), $P < 0.01$ (^^), and $P < 0.001$ (^^^).

Regarding tear production, the mean test strip wetting measurement was approximately 6 mm for Normal rats throughout the eight-week period, and 4 mm or less for T1D rats beginning on week 4 (Figure 2(b)). T1D-INS rats had reduced wetting measurements (i.e. dry eye) relative to Normal animals on weeks 5–8. Systemic treatment with NTX, and topical treatment with one drop of NTX, resulted in reduced tear production relative to Normals, but tear volumes significantly greater than T1D rats on weeks 5–8, suggesting that the magnitude of dry eye was diminished. Male T1D rats receiving two drops of NTX daily never developed dry eye over the course of eight weeks. Tear production for $T1D_{TopNTX\text{-}2drops}$ rats was recorded as approximately 5.8 mm over the course of eight weeks, and was comparable to Normal values and significantly different from levels recorded for T1D rats receiving saline. These data suggest that topical NTX twice daily protected against the onset of dry eye in diabetes.

A third diabetic corneal complication evaluated was wound size following a central corneal abrasion by assessment of residual re-epithelialization (Figure 3). T1D rats had residual wounds that were measurable for 96 h, whereas the wounds in Normal rats healed by 64 h preventing further statistical analyses. All rats receiving NTX had corneal abrasions that healed faster than T1D rats receiving saline (Figure 3(b)). The residual wound areas in rats

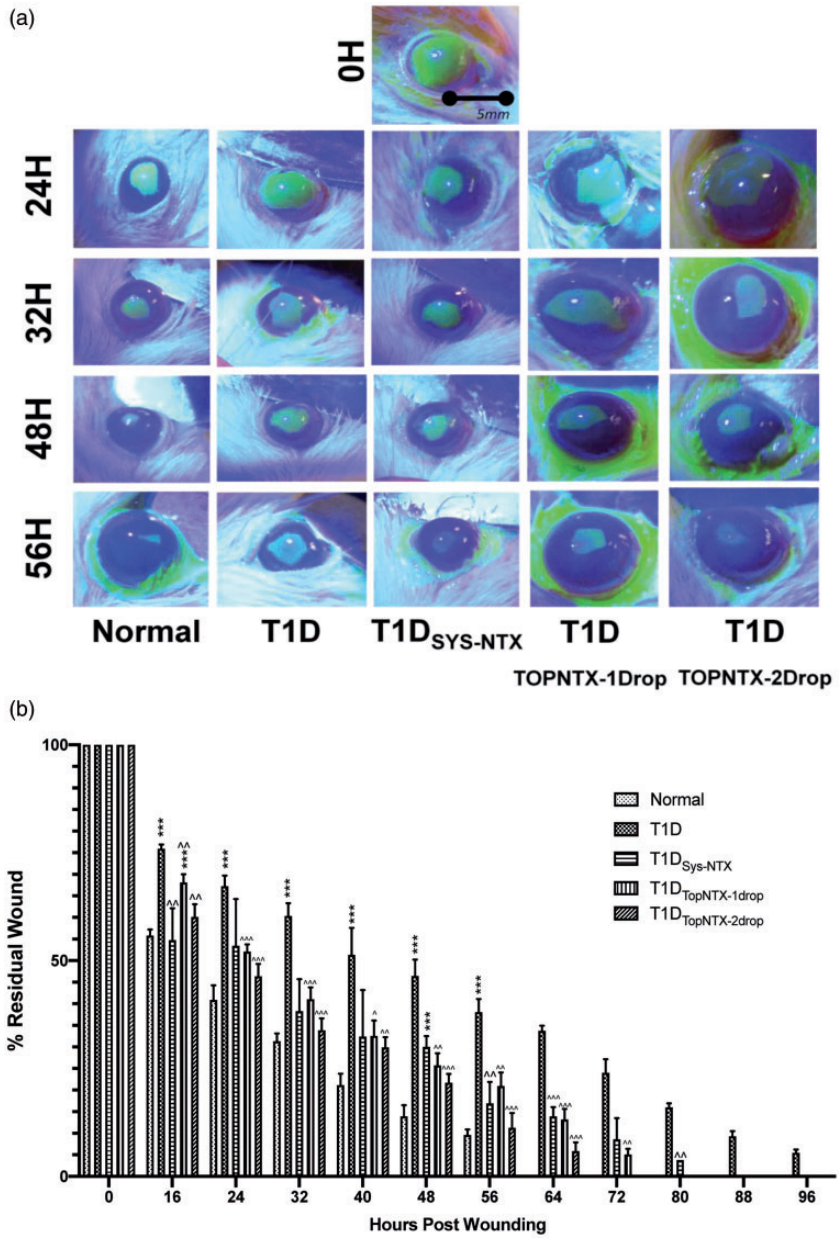

Figure 3. Residual corneal wounds in male T1D rats treated daily with NTX (see Figure 1 legend for explanation of groups); Normal rats were included for comparison. Photographs (a) of the fluorescein-stained ocular surface were collected at baseline (0 h), and 24, 32, 48, and 56 h after central corneal abrasion. Individual animals were photographed at intervals greater than 24 h such that each time point represents a random sampling. The residual corneal defect (b) was calculated from areal images for each rat at baseline (0 h), 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, and 96 h. Values represent means ± S.E.M. Significantly different from residual wound areas in Normal animals at $P < 0\ 05$ (*), $P < 0.01$ (**), $P < 0.001$ (***); significantly different from T1D residual areas at $P < 0.05$ (^), $P < 0.01$ (^^), and $P < 0.001$ (^^^).

treated topically were comparable to Normals beginning at 24 h. There were no measurable differences in the residual wound size rats receiving one drop of NTX topically either once or twice daily.

Dysregulated OGF and OGFr in corneal epithelium and serum

The mechanisms underlying some of these results remain unclear. Two factor ANOVA revealed a significant interaction between treatment groups and time ($P < 0.01$); subsequent ANOVA demonstrated that treatments differed from T1D and Normal levels at $P < 0.001$. OGF (Figure 4(a) and OGFr (Figure 4(b)) expression in corneal epithelium was significantly increased in T1D rats receiving either saline or systemic NTX, as well as those receiving one drop of NTX. However, corneal tissue treated twice daily with NTX revealed expression levels of both receptor and peptide that were comparable to Normal, non-diabetic rats.

Serum levels of OGF and OGFr were measured at four and eight weeks in Normal, T1D, $T1D_{NTX\text{-}Sys,}$ and $T1D_{NTX\text{-}Top\text{-}2\,drop}$ animals (Figure 5). Topical administration of NTX did not alter serum values of OGF, and levels were elevated from Normal values and comparable to those reported in T1D rats. However, systemic NTX significantly reduced serum OGF levels at both four and eight weeks, but not to levels recorded in non-diabetic rats. With regard to OGFr, values for all diabetic rats were significantly elevated relative to Normal at both four and eight weeks, and no differences were noted between NTX treatments.

Measurements of CD10, a peptidase that degrades OGF, were determined at four and eight weeks. CD10 levels were approximately 2 ng/μL at both time points for T1D and T1D-INS rats. These values were significantly less than Normal levels of 8 ng/μL and 4.5 ng/μL recorded at four and eight weeks, respectively.

NTX levels in the serum from rats treated systemically or topically were below detectable range of the kit (0.17 ± 0.02 to 0.23 ± 0.02 ng/mL).

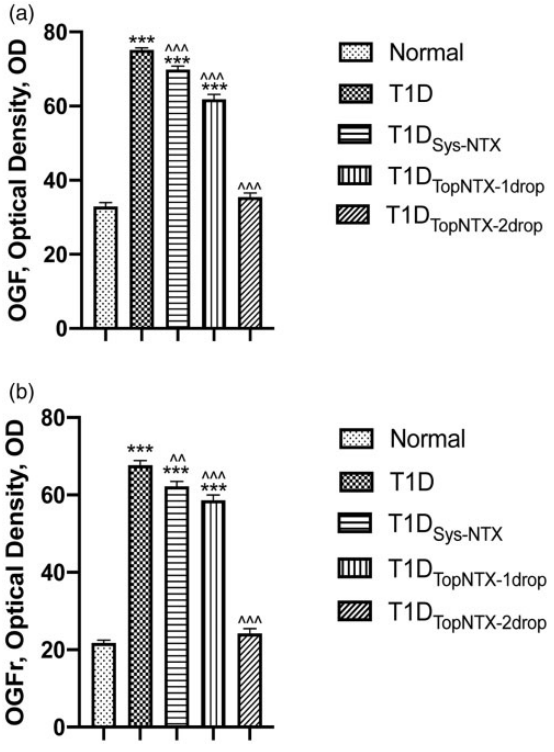

Figure 4. Intact corneas were stained with validated antibodies to OGF (a) and OGFr (b) at eight weeks post-STZ injection; groups are identified in the legend for Figure 1. Images were photographed at 10× magnification using confocal microscopy and optical density determined using ImageJ software. At least 15–20 optical density (OD) readings from tissue specimens from three different rats in each group were collected. Data were analyzed using one-factor ANOVA with post-hoc Newman–Keuls tests. Values represent means ± S.E.M. Significantly different from expression levels in Normal rats at $P < 0.001$ (***), and from T1D rats at $P < 0.01$ (^^) or $P < 0.001$ (^^^).

Discussion

Corneal-associated complications of diabetes have been recognized and studied by a number of laboratories.[6–9] In particular, corneal epithelialization has been reported to be enhanced by various growth factors included epidermal growth factor, insulin-like growth factor-1,[5,23,24] as well as blockade of growth factor interaction at the OGFr.[25] However, few of these therapies target an underlying biological system. Our laboratory has investigated the role of an active regulatory pathway involved in cell replication, the OGF–OGFr axis, as a pathophysiological cause of diabetic complications of the cornea. We have reported that blockade of this pathway with the antagonist NTX in Normal and diabetic rats and mice increased corneal epithelial cell proliferation during homeostasis, as well as following surgical abrasions of the corneal epithelium.[10–19] Because both systemic and topical therapeutic application of NTX was rapid and effective, the hypothesis was developed that one or more aspects of the OGF–OGFr axis may be dysregulated in diabetic animal models. Evidence of elevated serum levels of methionine enkephalin were corroborated by recent work demonstrating elevated serum and tissue levels in an animal model of T1D.[22] We hypothesized that the development of hyperglycemia/diabetes coincided with this dysregulation of serum and tissue levels of OGF and/or OGFr, and that the timing and magnitude of change in OGF and/or OGFr in diabetic male Sprague-Dawley rats corresponded to ocular surface complications in T1D.[22]

Data from the current investigations complement and extend this work,[22] and now show that blockade of the OGF interaction with OGFr by administration of NTX may delay, and even prevent, the onset of ocular surface complications. We previously reported that in male rats with T1D, serum levels of OGF and OGFr increased within four weeks of injection of STZ, implicating a strong association with hyperglycemia and elevated OGF and OGFr. The dysregulation of the OGF–OGFr pathway was further associated with changes in tear production and corneal surface sensitivity, as well as delayed re-epithelialization of corneal abrasions. Because OGF is an inhibitory growth factor, the elevated serum and tissue levels most likely reduce cell replication and thus contribute to the delayed corneal abrasions healing. Blockade of the OGF–OGFr pathway with NTX has been shown to be an effective treatment for delayed wound healing.[14,15,24] This study is the first to demonstrate that systemic or topical

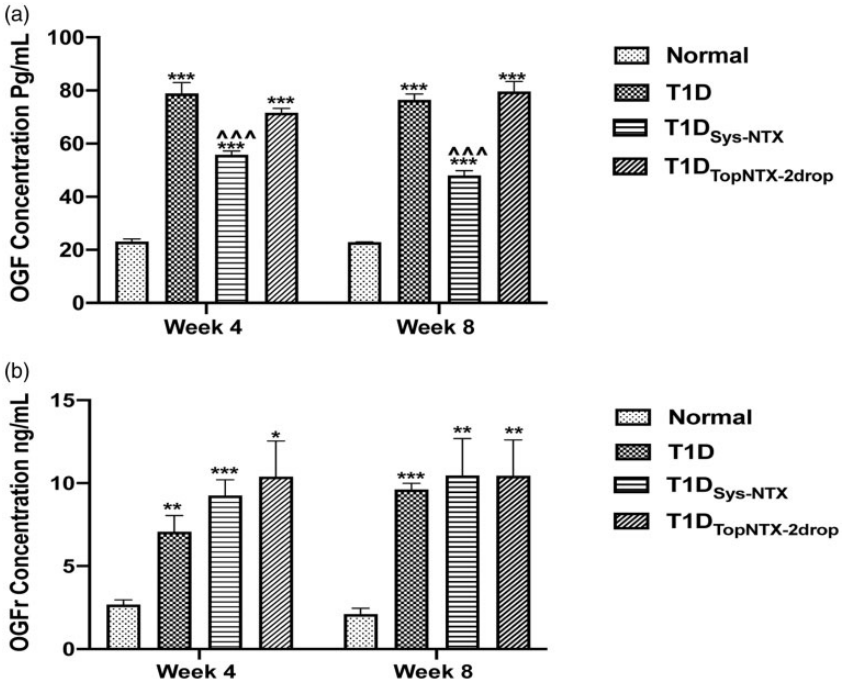

Figure 5. Serum levels of OGF (a) and OGFr (b) collected four and eight weeks post-STZ injections from rats in each group as described in the legend for Figure 1. Samples were assayed in duplicate using two or three different assays (ELISAs) from at least six different rats per group. Data were analyzed with a one-way ANOVA at each time point. Values represent means ± S.E.M. Significantly different from Normal values at $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***); significantly different from T1D levels at $P < 0.001$ (^^^).

administration of NTX prior to the onset of complications can delay and even prevent these complications from developing. Systemic or topical NTX administration did not reverse the body weight losses or elevated glucose levels reported in serum of T1D rats, whereas insulin implants were able to regulate these physiological characteristics. However, systemic NTX, injected daily, delayed the onset of dry eye and reduced the magnitude of dry eye throughout the eight weeks. Topical administration of NTX was more effective than systemic administration at ameliorating these abnormalities. Animals receiving twice daily drops of NTX never developed dry eye as reflected in decreased tear production. T1D rats receiving the single drop of NTX twice daily seemed to be protected against some diabetic-related ocular surface complications. Interestingly, systemic insulin did not protect, delay, or reduce the magnitude of reduced tear production or altered corneal surface sensitivity, suggesting that insulin does not aid in pathways involving neurological involvement including dry eye and corneal surface sensitivity.

With regard to the rate of re-epithelialization following a corneal abrasion, topical application of NTX twice daily was most effective at restoring the rate of wound closure, with no residual wound by 64 h, in comparison to approximately a 30% residual wound in the T1D group. Systemic NTX and once daily application of NTX also were capable of reducing the magnitude of defect and enhancing re-epithelialization. The changes observed following NTX application are likely correlated to decreases in the tissue expression or serum levels of the inhibitory growth factor OGF, as well as to the ability of OGF to interact with OGFr.

Although OGF and OGFr protein expression in the corneal epithelium were 2- to 3-fold increased in T1D rats relative to Normal rats, only the twice daily application of NTX was effective at reducing values to Normal. This effect is not surprising as systemic NTX is most likely not circulating to the corneal epithelium, and topical application of one-drop was inadequate to completely block the receptors on the epithelium. Thus, these applications often resulted in reductions in magnitude, but not "prevention" of complications.

Systemic treatment with NTX was most effective in changing the serum levels of OGF. These observations are expected because topical application most likely does not diffuse into the blood stream and circulate in sufficient quantity to have a whole body effect. However, when the changes in OGF expression following topical application are viewed in concert with serum-based effects of systemic NTX, there is strong evidence that OGF expression is correlated with ocular surface defects. As discussed earlier,[22] systemic insulin does not prevent or protect against dry eye and altered corneal surface sensitivity in T1D, whereas the OGF–OGFr pathway appears to have a significant role. In this study, it was demonstrated that two different methods of establishing receptor blockade of OGF from interaction with OGFr resulted in some level of protection from the ocular surface complications of diabetes. Moreover, the changes in tissue and serum levels of OGF corresponding to either topical or systemic administration support the concept that elevated OGF is causative of these complications although it may not be the only mechanism associated with diabetic-related ocular complications.

The causes for this dysregulation of the OGF–OGFr axis are currently understudied. The animal models of STZ-induced T1D, as well as the genetic mouse models of type 2 diabetes, are limited in their application to humans. STZ induction is the standard model for T1D study, but represents hyperglycemia in the short-term. Unfortunately, IACUC regulations prevent the long-term study of rats with T1D without insulin infusions, and even then, rats do not survive for extended periods of time. Nonetheless, the model we used in this study demonstrates diabetic complications comparable to those reported in humans, and the underlying pathophysiology of elevated OGF has been reported in humans with both type 1 and type 2 diabetes. Further investigations to study interactions between insulin and OGF in T1D are warranted in order to design prophylactic therapy for the millions of pre-diabetic and early diabetic individuals worldwide.

AUTHORS' CONTRIBUTIONS

All authors participated in the design and interpretation of the studies, and reviewed the final submission. IP conducted the experiments, PJM and IP analyzed data, PJM wrote the initial draft, PJM, ISZ, IP, and JWS contributed to editing the submitted manuscript.

DECLARATION OF CONFLICTING INTERESTS

PJM, ISZ, and JSW have intellectual property owned by Penn State Research Foundation and licensed to Ocunova, Inc. PJM, ISZ, and JSW have partial ownership in Ocunova Inc. but receive no financial compensation or royalties.

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