Dry Eye Reversal and Corneal Sensation Restoration With Topical Naltrexone in Diabetes Mellitus

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Objective: To determine if topical application of naltrexone hydrochloride (NTX), an opioid antagonist, restores tear production and corneal sensation in rats with diabetes mellitus.

Methods: Type 1 diabetes was induced with streptozotocin in rats. Tear production was measured by the Schirmer test, and corneal sensitivity, by an esthesiometer. Eye drops of 10^{-5} M NTX or sterile vehicle were administered either once only or 4 times a day for 1 or 5 days; a single drop of insulin (1 U) was given once only.

Results: Dry eye and corneal insensitivity were detected in the diabetic rats beginning 5 weeks after streptozotocin injection. One drop of NTX or 4 times a day for 1 or 5 days reestablished tear production and corneal sensitivity within 1 hour of administration. The reversal of dry eye lasted for up to 2 to 3 days depending on drug regi-

men, but restitution of corneal sensation lasted for 4 to 7 days. Topical application of 1 eye drop of insulin restored corneal sensitivity within 1 hour and lasted for at least 2 days. In contrast, 1 eye drop of insulin did not increase tear production at 1, 24, or 48 hours compared with diabetic animals receiving sterile vehicle.

Conclusion: Topical treatment with NTX normalizes tear production and corneal sensitivity in type 1 diabetic rats.

Clinical Relevance: Topical application of NTX to the ocular surface may serve as an important strategy for treating dry eye and corneal anesthesia in diabetes. Its effect, if any, in other forms of decreased corneal sensitivity and/or dry eye should be investigated.

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IABETES MELLITUS (DB) OFten is accompanied by dry eye disease, 1-3 with a significantly higher incidence reported in DB individuals than in non-DB individuals. 1,4,5 Not surprisingly, DB patients often complain of dry eye symptoms and may exhibit abnormal Schirmer test scores, blinking rate, basal tear production, tear film, and tear film breakup time, as well as differences in the composition of tear proteins, compared with healthy subjects. 2,4,6-9 Dry eye may be accompanied by ocular surface damage, alteration in the metabolism and vitality of the epithelium, and impairment of wound healing; may lead to ulceration; and, in general, can limit and degrade visual performance. 10,11 Although there are multiple modalities to treat dry eye disease (eg, punctal plugs, anti-inflammatory agents, topical tear and gel replacements), the etiology, management, and treatment of dry eye in DB remains a challenge to clinicians and researchers alike. 12,13

Naltrexone hydrochloride (NTX) is an opioid antagonist that has been reported to restore corneal reepithelialization and

prevent exuberant granulation tissue formation and corneal neovascularization in DB animals. 14-16 An important observation documented in studies on NTX in DB animals with respect to the cornea relates to the well-known complication of decreased sensitivity. 2,9 Topical NTX administered to DB rats in a wide range of concentrations restored corneal sensation. 17 The mechanism by which NTX acts to reestablish corneal sensitivity, or its effect, if any, in correcting dry eye, is unknown.

The present study examined the hypothesis that topical application of the opioid antagonist NTX restores tear production and corneal sensation in rats with type 1 DB.

METHODS

ANIMALS AND INDUCTION OF DB

Male Sprague-Dawley rats (approximately 245 g) were obtained from Charles River Laboratories (Wilmington, Massachusetts) and housed under standard laboratory conditions. ¹⁴⁻¹⁶ All investigations conformed to the regulations of the Association for Research in Vision and Ophthalmology and National Institutes of Health

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Type 1 DB was induced according to previously reported procedures. ¹⁴⁻¹⁶ Briefly, an intraperitoneal injection of 40 mg/kg of streptozotocin (STZ) (Sigma-Aldrich, St Louis, Missouri) in ice-cold, 0.5-mol/L citrate buffer (pH 4.5) was administered. A second dose of STZ (40 mg/kg) was injected 24 hours later. This regimen produced insulin (INS)-deficient DB in 100% of the animals within 48 to 72 hours; these animals were termed *DB rats* (n=25). Fifteen animals not receiving STZ, but injected with citrate buffer, were considered normal.

Blood glucose levels were monitored from the tail vein using a TrueTrack Smart System glucometer (Home Diagnostics, Inc, Ft Lauderdale, Florida) immediately prior to receiving STZ and at 1, 4, and 8 weeks after injection of STZ. Glucose levels of more than 400 mg/dL (to convert to millimoles per liter, multiply by 0.0555) were considered the minimum blood glucose level compatible with a stable nontoxic DB state. ¹²

All animals were numbered, and the investigators recording the measurements were masked to treatment.

SCHIRMER TEST

Tear secretion was measured with Schirmer strips (Alcon Laboratories, Inc, Ft Worth, Texas). A standard 17-mm-long Schirmer strip was inserted into the lower cul-de-sac for 1 minute. The strip wetting length was measured to the nearest millimeter. Five minutes prior to administration of the Schirmer strip, animals received topical proparacaine hydrochloride ophthalmic solution, 0.5% (Akorn, Inc, Buffalo Grove, Illinois). Testing began 1 hour after the last drop of NTX or vehicle was administered and continued every 24 hours thereafter.

CORNEAL SENSITIVITY

Corneal sensitivity was determined by an esthesiometer (Cochet and Bonnet, Boca Raton, Florida), with the end point of blinking; 4 measurements were taken for each animal and averaged. The values were determined directly from the protocol (and conversion table) supplied by the manufacturer. Measurements of sensitivity were conducted prior to the Schirmer test.

SLITLAMP OBSERVATIONS

To examine general overall morphology and pathology (eg, corneal edema, scarring), observations with a handheld slitlamp (HSO 10 Hand Slit Lamp; Zeiss, Dublin, California) were conducted.

TOPICAL ADMINISTRATION OF NTX

Naltrexone hydrochloride (Sigma-Aldrich, Indianapolis, Indiana) was prepared in moxifloxacin hydrochloride ophthalmic solution (Vigamox; Alcon, Inc) at a 10^{-5} M concentration. Naltrexone hydrochloride was given as a single drop (0.05 mL) to the central cornea of the right eye, with the lower eyelid held away from the eye to avoid overflow. Naltrexone hydrochloride or vehicle was administered once at 7 AM or at 7 AM, 11 AM, 2 PM, and 5 PM for 1 or 5 days, beginning on the ninth week after induction of DB.

TOPICAL ADMINISTRATION OF INS

Bovine INS (Sigma-Aldrich, Indianapolis) was prepared in moxifloxacin hydrochloride ophthalmic solution (Vigamox; Alcon, Inc) and used at a concentration of 1 U, with a single drop administered to the central cornea of the eye, as described in a previous report. ¹⁶ Insulin or vehicle was applied topically at 8 AM.

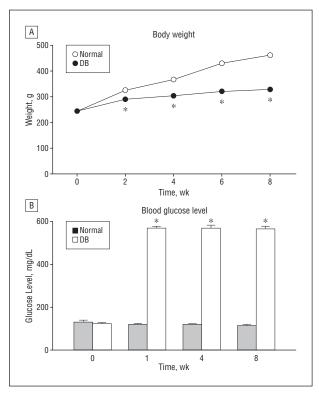


Figure 1. Body weights (A) and glucose levels (B) of rats rendered diabetic (DB) with streptozotocin or untreated animals receiving sterile vehicle (normal). A, Body weights were recorded at the time of streptozotocin injection (week 0) and every 2 weeks thereafter. B, Blood glucose levels were recorded 1, 4, and 8 weeks after administration of streptozotocin. Data are expressed as mean (SEM) for 15 normal and 25 DB animals at each point. *Significantly different from normal rats at P < .001. To convert glucose to millimoles per liter, multiply by 0.0555.

DATA ANALYSIS

For body weights and glucose measurements, 2-tailed *t* tests were used. With Schirmer scores and esthesiometer measurements, 1-way analysis of variance with Newman-Keuls posttests was used because measurements were conducted on a random sample of two-thirds of the rats in each group.

RESULTS

INDUCTION OF DB

All rats weighed mean (SEM) 245 (6) g at the time of STZ injections (**Figure 1**A). Normal rats gained approximately 216 g over the course of 8 weeks. Rats in the DB group were comparable in body weight with normal animals until 2 weeks after injection of STZ. At this time, the DB group had a 10% reduction (P<.001) in body weight relative to normal animals. In subsequent weeks, the DB rats weighed significantly less (approximately 17%-29%) than normal rats throughout the course of the 8-week study.

Baseline mean (SEM) glucose readings were 131 (8) mg/dL for all rats (Figure 1B), and these values were maintained throughout the study in the normal group. Rats receiving STZ became hyperglycemic within 5 days (Figure 1B) and had glucose levels greater than 520 mg/dL throughout the duration of experimentation.

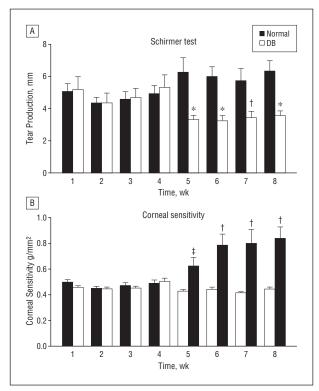


Figure 2. Tear production (A) and corneal sensitivity (B) were measured at weekly intervals after streptozotocin injection. Data are expressed as mean (SEM) for 15 normal and 25 diabetic (DB) animals at each point. *Significantly different from normal rats at P<.001. †Significantly different from normal rats at P<.01. ‡Significantly different from normal rats at P<.01. *Significantly different from normal rats at P<.01. *Significantly different from normal rats at P<.01.

TEMPORAL COURSE OF TEAR PRODUCTION AND CORNEAL SENSITIVITY

Tear production as measured by the Schirmer test (**Figure 2**A) and corneal sensitivity as determined with an esthesiometer (Figure 2B) in DB rats were comparable with normal animals for the first 4 weeks after injections with STZ. Beginning on week 5, and continuing thereafter, the DB rats had a decrease of 40% to 47% in the Schirmer score and a 1.5- to 1.9-fold reduction in corneal sensitivity.

TEAR PRODUCTION AND TOPICAL NTX TREATMENT

Topical administration of 1 drop of NTX restored tear secretion to the DB rat within 1 hour (**Figure 3**A). Normal and DB NTX rats had comparable Schirmer scores, whereas the DB sterile vehicle (SV) rats exhibited basal tear production that was more than 39% less than the other groups. Tear production for the DB rats subjected to 1 drop of NTX was similar to normal animals for at least 48 hours after administration. However, the DB SV rats' tear production was 29% to 43% less than the normal and DB NTX rats at both 24 and 48 hours.

Diabetic rats receiving topical treatment with NTX for 1 (**Figure 4**) or 5 (**Figure 5**) days 4 times a day had Schirmer scores that were comparable with normal rats beginning 1 hour after termination of drug exposure and extending for at least 3 days thereafter (Figure 4 and

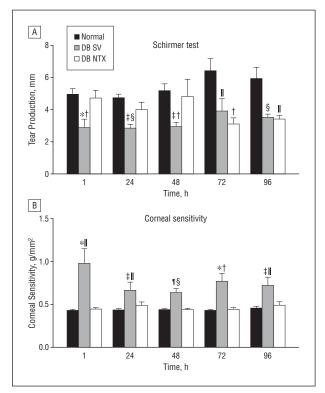


Figure 3. Tear production (A) and corneal sensitivity (B) of diabetic (DB) rats given 1 drop of topical naltrexone hydrochloride (NTX) or sterile vehicle (SV) as well as untreated animals given SV (normal) to the cornea. Data are expressed as mean (SEM) for 5 to 10 rats per group at each point. *Significantly different from DB rats receiving NTX at P<.01. †Significantly different from normal rats at P<.01. ‡Significantly different from DB rats receiving NTX at P<.05. §Significantly different from normal rats at P<.01. ||Significantly different from normal rats at P<.05. ¶Significantly different from DB rats receiving NTX at P<.05. ¶Significantly different from DB rats receiving NTX at P<.001.

Figure 5). However, the DB animals receiving SV had tear production scores that were 32% to 53% less than both the normal and DB NTX groups. By 96 hours after termination of either 1 or 5 days of NTX administration (4 times a day), the effects of NTX had attenuated so that tear production in the DB NTX group was 22% to 59% less than normal animals and similar to levels of tears in the DB SV group. Measurements of the Schirmer test scores for the DB NTX group remained significantly reduced compared with normal animals for the duration of the experiment.

CORNEAL SENSITIVITY AND TOPICAL NTX TREATMENT

Topical administration of 1 drop of NTX restored corneal sensitivity in DB rats within 1 hour (Figure 3B) and extended for the entire 96-hour period of the experiment, with normal and DB NTX rats having comparable measurements. However, the DB SV rats' corneal sensitivity was 1.4- to 1.8-fold less than the normal and DB NTX rats over the entire 96-hour course of the study.

In contrast, DB rats receiving topical treatment with NTX for 1 or 5 days 4 times a day had corneal sensitivity scores that were comparable with normal rats beginning 1 hour after termination of drug exposure and extending for at least 4 days thereafter (Figure 4B and Figure 5B). However, the DB animals receiving SV had sensitivity scores

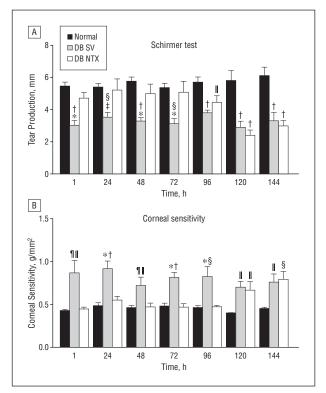


Figure 4. Tear production (A) and corneal sensitivity (B) of diabetic (DB) rats given topical naltrexone hydrochloride (NTX) or sterile vehicle (SV) as well as untreated animals subjected to SV (normal) for 1 day (4 times per day) to the cornea. Data are expressed as mean (SEM) for 6 to 11 animals per group at each point. *Significantly different from DB rats receiving NTX at P<.001. †Significantly different from normal rats at P<.001. ‡Significantly different from normal rats at P<.05. ¶Significantly different from DB rats receiving NTX at P<.05.

that were 1.5- to 2.0-fold less than both the normal and DB NTX groups. At 120 hours after termination of the 1 day of treatment with NTX (4 times a day), the NTX effect had expired so that the sensitivity in the DB NTX group of rats was 1.8-fold less than the normal animals and similar to the levels of sensitivity in the DB SV group. At 192 hours after termination of the 5-day treatment with NTX (4 times a day), sensitivity in the DB NTX group was 1.9-fold less than the normal animals and comparable with the DB SV group. At 216 hours after termination of the 5-day regimen of NTX, corneal sensitivity of the DB NTX group remained 1.7-fold less than the normal animals.

NONINVASIVE MEASUREMENTS OF CORNEAL INTEGRITY

Examination with a handheld slitlamp during and after NTX administration did not reveal any abnormalities of the ocular surface in any animal of any group.

TEAR PRODUCTION AND CORNEAL SENSITIVITY: TOPICAL INS TREATMENT

Topical administration of 1 drop of INS had no effect on tear production in DB rats (**Figure 6**A). The Schirmer score for the DB INS group was 35% to 58% less than normal rats at the 1-, 24-, and 48-hour points, whereas the DB SV

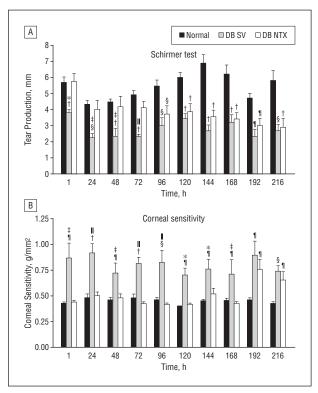


Figure 5. Tear production (A) and corneal sensitivity (B) of diabetic (DB) rats given topical naltrexone hydrochloride (NTX) or sterile vehicle (SV) as well as untreated animals subjected to SV (normal) for 5 days (4 times per day) to the cornea. Data are expressed as mean (SEM) for 5 to 10 rats per group at each point. *Significantly different from DB rats receiving NTX at P<.01. †Significantly different from normal rats at P<.001. ‡Significantly different from DB rats receiving NTX at P<.01. |Significantly different from DB rats receiving NTX at P<.01. |Significantly different from DB rats receiving NTX at P<.001. ¶Significantly different from normal rats at P<.05.

group scores were 27% to 61% lower. Thus, the values for the DB INS and DB SV groups were comparable.

Corneal sensitivity of the DB rats receiving topical INS did not differ from the normal animals at any point (Figure 6B). However, the rats treated with SV had reductions in esthesiometer scores ranging from 1.3- to 1.8-fold less than normal subjects and 1.1- to 1.9-fold less than the DB INS group. Corneal sensitivity for normal and DB INS rats was comparable at the 1-, 24-, and 48-hour points.

COMMENT

The present study makes the novel observation that topical NTX treatment in DB rats restores tear production and confirms and extends previous reports¹⁷ that exposure to this opioid antagonist reestablishes corneal sensation. Inspection of the temporal course of DB revealed that tear production and corneal sensitivity were comparable with normal animals for at least 4 weeks after induction of the disease. However, on week 5 after the induction of DB, both Schirmer scores and esthesiometer results documented the appearance of dry eye and a marked decrease in corneal sensitivity, respectively. Moreover, these abnormalities were not transient but remained throughout the course of the experiment in untreated DB rats (ie, DB SV group). Our studies reveal that treatment with even 1 drop of 10⁻⁵M NTX restores both tear production and cor-

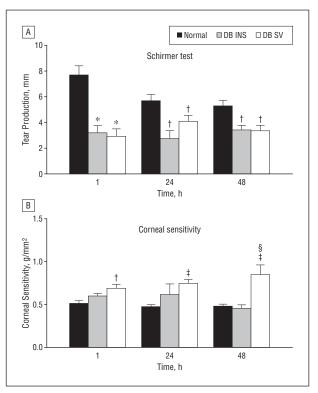


Figure 6. Tear production (A) and corneal sensitivity (B) of diabetic (DB) rats given 1 drop of topical insulin (INS) naltrexone hydrochloride or sterile vehicle (SV) as well as untreated animals subjected to SV (normal) to the cornea. Data are expressed as mean (SEM) for 5 to 10 rats per group at each point. *Significantly different from normal rats at P<.001. †Significantly different from DB rats receiving INS at P<.05.

neal sensitivity to levels similar to normal rats. This result was detected within 1 hour of NTX administration, thereby indicating an extraordinarily rapid onset of action of this opioid antagonist. The normalization of tear production and corneal sensation extended for at least 2 days after NTX treatment. Although dry eye reappeared between 48 and 72 hours, normal corneal sensitivity remained for at least 96 hours. A more extensive treatment regimen with either 4 or 20 drops of topical NTX over a 1- or 5-day period again showed that within 1 hour of the last eye drop of NTX (10⁻⁵M), normalization of both tear production and corneal sensitivity were recorded in DB animals, but in contrast to 1 drop of NTX, the effect lasted for at least 72 hours. As in the case of 1 drop of NTX, dry eye reappeared considerably earlier than the loss of corneal sensation, with normal corneal sensitivity lasting at least 1 day longer with the 4-drop regimen and at least 6 days longer for the 20drop regimen. Thus, to our knowledge, we have shown for the first time that treatment with topical 10⁻⁵M NTX can normalize tear production in type 1 DB animals. Future studies are needed to define whether topical NTX also will be an effective treatment for dry eye in type 2 DB.

Both a loss of corneal sensitivity and tear production have been recorded previously in DB patients and animals.^{28,9} Based on our finding that loss of corneal sensitivity and tear production had an onset at the same time in DB rats, and that they could be restored simultaneously, one could speculate that these 2 entities are interrelated. However, at least 2 lines of evidence in the present

study suggest that corneal sensation and tear production are not entirely related. First, topical application of NTX, whether 1 drop or for 1 or 5 days (4 times a day), consistently revealed that dry eye reappeared at least several days prior to corneal insensitivity. Thus, it would be incorrect to postulate that restoration of tear production was due to a reemergence of corneal sensitivity because reestablishment of corneal sensation lasted far beyond that of restoration of tear production. Second, it is known¹⁶ that topical INS can restore corneal sensitivity in animals with type 1 DB, and this fact was confirmed in the present study. To test the possibility that restoration of corneal sensitivity also recovers tear production, DB rats were given topical INS and underwent a Schirmer test. Dry eye was recorded at 1, 24, and 48 hours after administration of 1 drop of INS, despite the fact that normal corneal sensitivity was present continuously. Therefore, at least in type 1 DB, corneal sensitivity does not appear to be causally related to increased tear production. These results support the findings of Inoue and colleagues,7 who found that a reduced blinking rate in DB patients was not correlated with such ocular surface factors as corneal sensitivity, tear breakup time, or the status of the lipid layer. Moreover, Saito et al¹⁸ have reported no correlation of corneal sensitivity with tear secretion with regard to the stage of diabetic retinopathy.

The mechanisms underlying dry eye and corneal insensitivity in type 1 DB are unknown. Some investigators have speculated that dry eye results from dysfunction of the integrated ocular surface-secretory glandular functional unit. 19 This dysfunction may result from peripheral neuropathy, with disease of the sensory afferent nerves innervating the ocular surface, the autonomic (efferent) nerves innervating the tear-secreting glands, and/or the tearsecreting glands themselves. 23,9,19-21 Additional information shows that inflammation, whether a cause or effect or both, frequently accompanies dry eye in rodents and humans and may represent a potential mechanism in the etiology and pathogenesis of this disease.^{22,23} At least in the case of type 1 DB, we now have demonstrated that the causative factor(s) for both dry eye and a loss of corneal sensation are corrected within 1 hour of application of NTX. This finding implies that whatever is altering tear production and corneal sensitivity in the DB cornea is reversible, although it takes at least 4 weeks for the damaging influence(s) to be expressed.

Because we used topical anesthesia prior to measuring tear production, we did not evaluate reflex response from the main lacrimal gland, but rather basal secretion from other sources, such as the accessory lacrimal glands of Krause and Wolfring. 24,25 These glands are said to be supplied by postganglionic, parasympathetic nerve fibers (of pterygopalatine ganglion origin) carried by the nervus intermedius from the VII cranial nerve. 26-28 Given the rapid reversal of dry eye, as well as corneal insensitivity, it may be proposed that elevated opioid levels, known to occur in DB individuals^{29,30} and which have been identified in corneal nerves,31 diminish the responsiveness of peripheral sensory or motor nerves innervating the ocular surface and/or accessory lacrimal glands. Naltrexone hydrochloride, a well-known general opioid antagonist, may be postulated to interfere with opioid-opioid receptor interactions, thereby restoring tear production and corneal sensitivity. Surprisingly, even 1 drop of NTX may last for several days in terms of restoring function. Future studies should be directed at determining which opioid peptide(s) and receptor(s) are determinants of tear production and corneal sensation. Moreover, dry eye and a loss of corneal sensation are multifactorial disorders, ³² and exploration of the effectiveness of NTX in the face of diseases other than DB is warranted.

The present study documents reversal of dry eye and restoration of corneal sensation in DB animals by an opioid antagonist. It represents a paradigm shift involving the etiology, pathogenesis, and treatment of these 2 DB complications. Strategies for therapy involving interference with opioid-opioid receptor interfacing, as well as investigation into the origin and pathobiology of these complications resulting from opioid dysfunction, are warranted. Meanwhile, even 1 drop of NTX immediately restores corneal sensitivity and tear production in DB rats, and the effects can last up to 2 to 3 days. This would suggest the need for the initiation of clinical trials examining the safety and efficacy of this drug for ocular use. Moreover, it may be reasonable to evaluate the impact of NTX on other nonocular complications of DB, such as peripheral neuropathy, and its role, if any, in the treatment of other disorders of corneal sensitivity and/or tear production.

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