Role of Nicergoline in Corneal Wound Healing in Diabetic Rats

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Research Article

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Abstract

Background: To investigate the effect of nicergoline on the rate of complete corneal ulcer reepithelialization (CCUR) in diabetic rats with diabetic keratopathy.

Methods: Forty-eight streptozotocin-induced diabetic rats were randomly divided into two groups. The experimental group (n=24) received nicergoline (10 $mg.kg^{-1}.day^{-1}$), while the control group (n=24) received a placebo. A corneal epithelial defect was induced using a corneal diamond burr, and defect area was compared at timepoints of 0, 12, 24, 48 and 72 hours after the injury using image analysis software. The probability of CCUR within 72 hours was assessed using the Kaplan–Meier survival analysis log-rank test.

Results: When compared, 4 of the 24 rats (17%) in the placebo group and 12 of the 24 rats (50%) in the nicergoline group were found to have CCUR within 72 hours (log-rank = 0.027). Cox regression analysis found no effect of the covariates blood glucose (P=0.601) or weight (P=0.322) on the corneal reepithelialization (survival) curve.

Conclusions: Nicergoline increased wound healing rates relative to placebo and may therefore be investigated as a treatment option in diabetic keratopathy.

Background

Diabetes affects 451 million people worldwide, approximately 70% of whom suffer from corneal complications known collectively as diabetic keratopathy. The most frequent clinical signs of diabetic keratopathy include punctate keratitis, recurrent epithelial erosions, and ulcers that are refractory to conventional treatments and which often fail to heal completely.[1]

Previous controlled studies with corneal epithelial cell cultures in medium with high glucose levels have revealed that hyperglycemia over a period of 48 hours is able to delay corneal reepithelialization to levels of only 29% of normal and to greatly increase the production of reactive oxygen species.[2] The pathogenesis of diabetic keratopathy is multifactorial; prior studies have pointed to the impairment of the phosphatidylinositol 3-kinase (PI3K/AKT) transduction pathway, which responds to cell proliferation and migration in various systems and which also affects corneal healing.[2] In addition, oxidative stress and inflammatory cytokine production generated by hyperglycemia greatly contribute to the induction of cellular injury.[2,3]

In a previous study, rats with streptozotocin-induced diabetes exhibited a significant reduction in subepithelial basal nerve plexus density, as well as delayed closure of corneal epithelial ulcers 8 weeks after diabetes induction relative to controls.[4]

Certain growth factors, cytokines, and neurotrophins are fundamental to corneal wound healing and act as regulators of cellular behavior and corneal epithelial regeneration. These include epidermal growth

factor (EGF), fibroblast growth factor (FGF), substance P (SP), nerve growth factor (NGF), insulin-like growth factor-1 (IGF-1), and acetylcholine.[5,6] New drugs targeting such factors have been investigated to treat diabetic keratopathy, since diabetes is associated with a significant decrease in the levels of these molecules.[7,8,9,10,11,12]

Nicergoline (10a-methoxy-1,6-dimethylergoline-8β-methanol-5- bromonicotinate, Sermion, Biogenesis AntiAging, Fish Hoek, South Africa) is an ergoline derivative indicated for cerebrovascular dementias with a broad mechanism of action. It acts as an a-1 adrenergic antagonist, acetylcholinesterase inhibitor, dopaminergic agonist and PI3K/AKT pathway activator; it also increases SP and NGF levels and exhibits antioxidant properties. Such properties have inspired research into its use in corneal wound healing.[13,14,15,16,17,18]

In a previous study, nicergoline contributed to the corneal reepithelialization of 110 eyes from 100 rats, and corneal NGF protein was higher in the nicergoline-treated group than in the control group.[16] In another experimental study that assessed 10 eyes from 5 healthy dogs without a control group, the effect of nicergoline on the ocular surface was assessed using corneal esthesiometry, Schirmer's test 1, and tear film break-up time, but the drug did not significantly alter canine ocular surface parameters.[19]

In a prospective, noncomparative interventional study that included 27 eyes from 24 patients with neurotrophic keratopathy of multiple causes who had been unresponsive to conventional therapy, treatment with nicergoline contributed to the healing of neurotrophic corneal ulcers in 83% of eyes and improved Cochet–Bonnet corneal sensitivity and best-corrected visual acuity. In addition, tear NGF levels were significantly higher after nicergoline treatment.[20]

Methods

Radioisotope-marked nicergoline reaches peak serum radioactivity 3 hours after oral administration. Its bioavailability is 5% of the total dose administered due to the first-pass effect, and it is predominantly eliminated in the urine. On average, 82% of marked nicergoline is eliminated in the urine and 10% in the feces as of 120 hours after administration.[18,22]

Because the dosage administered to animals was 10 $mg.kg^{-1}.day^{-1}$ and because the bioavailability of nicergoline is only 5%, a loading dose of nicergoline was administered for the 15 days prior to surgery in order to maintain high serum nicergoline levels for an extended period. Nicergoline administration 15 days before corneal wound procedure was performed as described in similar studies.[16,18,22]

Descriptive statistic data were calculated. The Kolmogorov–Smirnov normality test was used for continuous data. Means and standard deviations (SDs) were used for normally distributed data, and medians and interquartile ranges (IQR) were used for non-normally distributed data. Between-group differences in continuous variables were compared using the independent samples t-test for normally distributed data or using the Mann–Whitney U-test for non-normally distributed data.

The probability of reach complete corneal ulcer reepithelialization (CCUR) within 72 hours was assessed using the Kaplan–Meier survival analysis log-rank test. Multivariate survival analysis was performed using the Cox regression model. The analyses were conducted using SPSS, version 21 (IBM Corporation, Armonk, NY, USA). P-values were two-tailed, and statistical significance was set at 0.05.

Results

Demographic data are displayed in Table 1 and demonstrate homogeneity of the two groups in terms of weight and blood glucose.

Table 1. Demographic data of the diabetic rats in the placebo and nicergoline groups.

Clinical Value	Placebo (n=24)	Nicergoline (n=24)	P-Value
Blood glucose,[a] mean (SD[c]), median (IQR[d]), mg/dL	351 (81), 308 (156)	314 (66), 309 (95)	0.085 [b]
Weight,[a] mean (SD), median (IQR), g	230 (39), 230 (68)	247 (34), 250 (53)	0.103 [b]

[a]normally distributed data; [b]independent samples t-test, [c] standard deviation, [d]interquartile range

The analyses revealed that 4 of the 24 rats (17%) in the placebo group and 12 of the 24 rats (50%) in the nicergoline group achieved complete reepithelialization of the cornea within 72 hours (log-rank = 0.027). Cox regression analysis showed no influence of covariates on the survival curve of the corneal reepithelialization variable. The covariates assessed were glycemia (P=0.601), weight (P= 0.322), and duration of diabetes (P=0.208).

Table 2 details corneal ulcer reepithelialization progress. The area of the corneal ulcer at the 0-hour time interval (immediately after surgery) was found to be consistent between the two groups: 6.42 ± 1.17 mm^2 in the placebo group and 6.07 ± 1.31 mm^2 in the nicergoline group (P=0.330).

Table 2. Corneal reepithelialization in diabetic rats that underwent mechanical de-epithelialization after treatment with nicergoline or placebo.

Measurement time	Epithelial defect area, mean (SD[c]), median (IQR),[d] mm^2		P-Value
	Placebo	Nicergoline	
0 h [a]	6.42 (1.17), 6.20 (1.28)	6.07 (1.31), 6.07 (1.27)	.330 [e]
12 h [a]	5.69 (0.99), 5.40 (0.78)	4.71 (1.53), 4.68 (2.51)	.018 [e]
24 h [a]	4.52 (1.56), 4.45 (2.36)	3.15 (1.65), 3.30 (3.08)	.012 [e]
48 h [b]	3.57 (3.21), 1.67 (4.52)	2.07 (1.41), 0.50 (2.00)	.095 [f]
72 h [b]	3.51 (3.48), 0.97 (4.38)	1.24 (0.87), 0.00 (0.72)	.017 [f]

[a]normally distributed data, [b]non-normally distributed data, [c]standard deviation, [d]interquartile range, [e]independent samples t-test, [f]Mann–Whitney U-test.

Table 2 also shows that the area of the corneal ulcer was found to be significantly smaller in the nicergoline group at various time intervals. At the 12-hour time interval, the area of the corneal ulcer in the nicergoline group (4.71 ± 1.53 mm^2) was smaller than that of the placebo group (5.69 ± 0.99 mm^2; P=0.018).

The ulcers in the experimental group were also smaller at the 24-hour time interval: 3.15 ± 1.65 mm^2 in the nicergoline group compared to 4.52 ± 1.56 mm^2 in the placebo group (P=0.012).

The findings were consistent at the 72-hour time interval as well, at which point the area of the corneal ulcer in the nicergoline group (1.24 ± 0.87 mm^2) was considerably lower than that of the placebo group (3.51 ± 3.48 mm^2; P=0.017).

Figure 1 illustrates the corneal reepithelialization process in the two groups. Figure 2 shows an example of 1% fluorescein staining of the corneal epithelial defect in the nicergoline and placebo groups immediately after surgery and at the 12-, 24-, 48-, and 72-hour time intervals thereafter.

Figure 1. Corneal reepithelialization progress in diabetic rats along the time interval considered in eyes treated with nicergoline or placebo.

Figure 2. Images of fluorescein corneal staining showing the area of epithelial defect in green along the time interval considered in eyes treated with nicergoline or placebo.

The median and interquartile range (IQR) of the reepithelialization rate in the first 72 hours was 0.04 (0.06) mm^2/h in the placebo group and 0.08 (0.03) mm^2/h in the nicergoline group (P=0.055).

Discussion

The present study relied on an experimental model that reproduced insulin-dependent diabetes type 1. The animals presented epithelial defects that failed to heal completely, thus resulting in the corneal ulcers commonly seen in cases of diabetic keratopathy.

Reepithelialization occurred twice as quickly in the experimental group as in the control group. Similar studies have found a 1.55-fold increase in mean reepithelialization speed using a fibronectin-derived peptide eye drop treatment and a 1.47-fold increase using SP with insulin-like growth factor-1 eye drop treatment.[23]

The two groups were also compared in terms of their ability to reach CCUR. Nicergoline was associated with significantly more corneal ulcer healing: after 72 hours, 50% of the nicergoline group presented CCUR, while only 17% the of placebo group exhibited the same outcome (log-rank = 0.027). Previous studies have also reported a delay in the reepithelialization of corneal defects in diabetic rats and a persistence of unhealed epithelial defects after 72 hours, and, in other studies, after more than 120 hours.[10,24]

The nicergoline group exhibited a statistically significant decrease in the area of the corneal ulcer at the 12-hour, 24-hour, and 72-hour time intervals. This finding suggests that nicergoline promotes corneal epithelial healing in diabetic rats and may be considered a promising new treatment option worthy of further investigation.

To date, this is the first experimental, controlled, and double-blind study to investigate the potential of nicergoline to improve the rate of corneal reepithelization and to treat corneal ulcers in a clinical context of diabetes, since the other studies in the literature evaluated this drug only in healthy animals.[13,14,19]

The current study exhibits some limitations. It is important to mention the lack of proof of diabetic neuropathy by an objective test, such as the corneal sensitivity test with the Cochet–Bonnet esthesiometer. We opted to forgo this procedure due to its low sensitivity and reproducibility.[25,26] A second limitation was the type of intervention used (nicergoline vs. placebo) prior to corneal surgical injury. The nicergoline dosing regimen used in this study ($10mg.kg^{-1}.day^{1}$) administered 15 days before the corneal wounding procedure was similar to the methodology used in previous studies and thus seemed to be appropriate.[16] Indeed, post-treatment serum SP and NGF levels in the corneal tissue of the animals were not measured, but as described previously, an increase in SP and NGF in corneal tissue after nicergoline treatment has been previously observed in healthy animals.[16,17]

Conclusions

In conclusion, nicergoline was found to be associated with corneal wound healing in diabetic rats and likely contributed to the outcomes found herein. The rate of complete corneal ulcer reepithelialization after 72 hours was higher among diabetic rats receiving nicergoline than in the placebo group. This

evidence is promising, and more studies are needed to evaluate nicergoline as a potential treatment of corneal ulcers secondary to diabetic keratopathy.

Abbreviations

- CCUR – complete corneal ulcer reepithelialization
- PI3K/AKT - phosphatidylinositol 3-kinase AKT pathway
- EGF - Epidermal growth factor
- FGF - Fibroblast growth factor
- SP - Substance P
- NGF - Nerve growth factor
- IGF-1 - Insulin-like growth factor-1
- CEUA - Research Ethics Committee on Animal Research
- UFPE - Federal University of Pernambuco
- CONCEA - National Council for the Control of Animal Experimentation
- LIKA - Keizo-Asami Immunopathology Laboratory
- OS - left eye
- SDs - standard deviations
- IQR - interquartile range

Declarations

Ethics approval and consent to participate

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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Figures

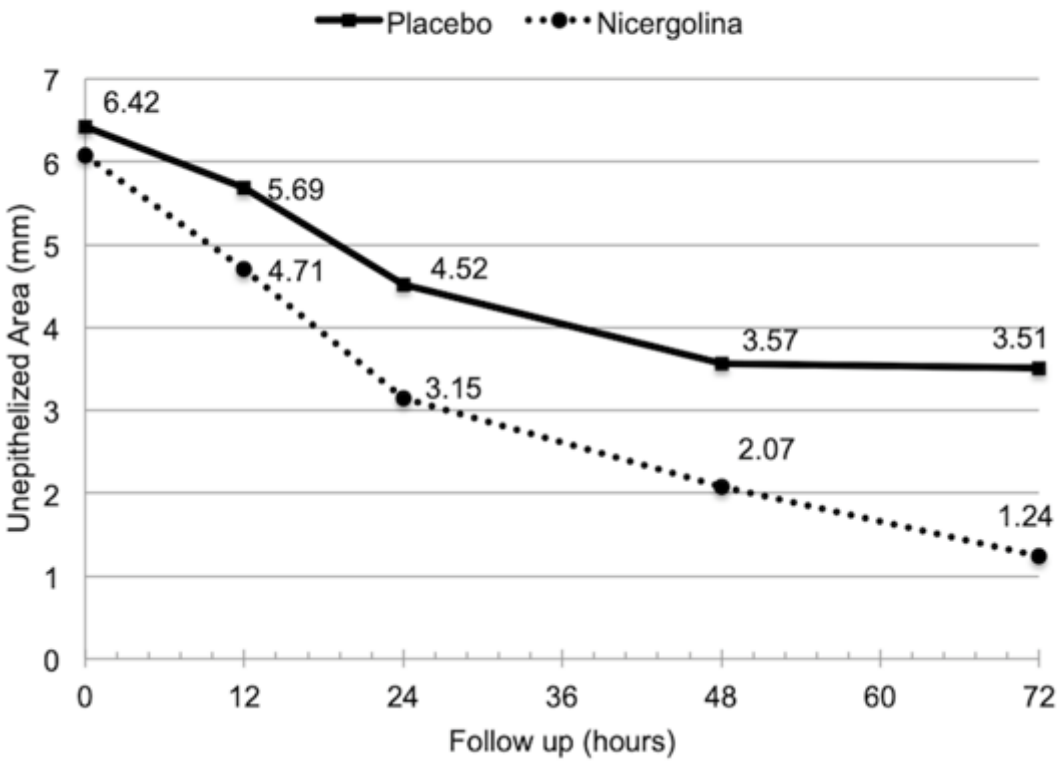
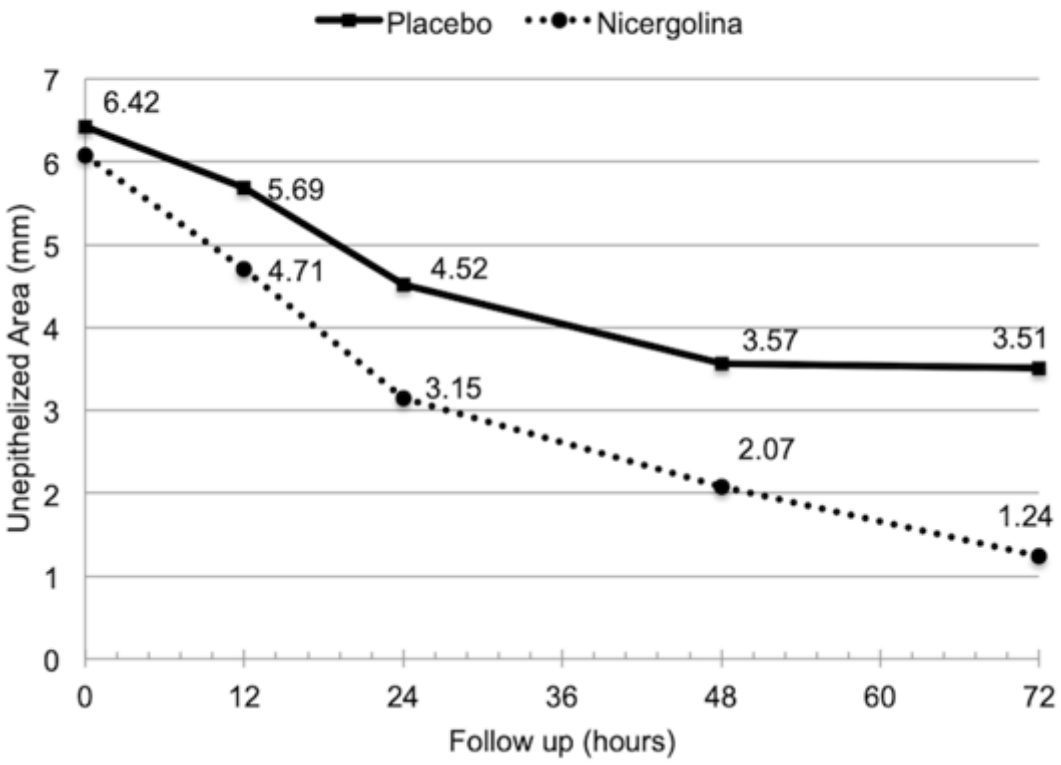


Figure 1

Corneal reepithelialization progress in diabetic rats along the time interval considered in eyes treated with nicergoline or placebo.



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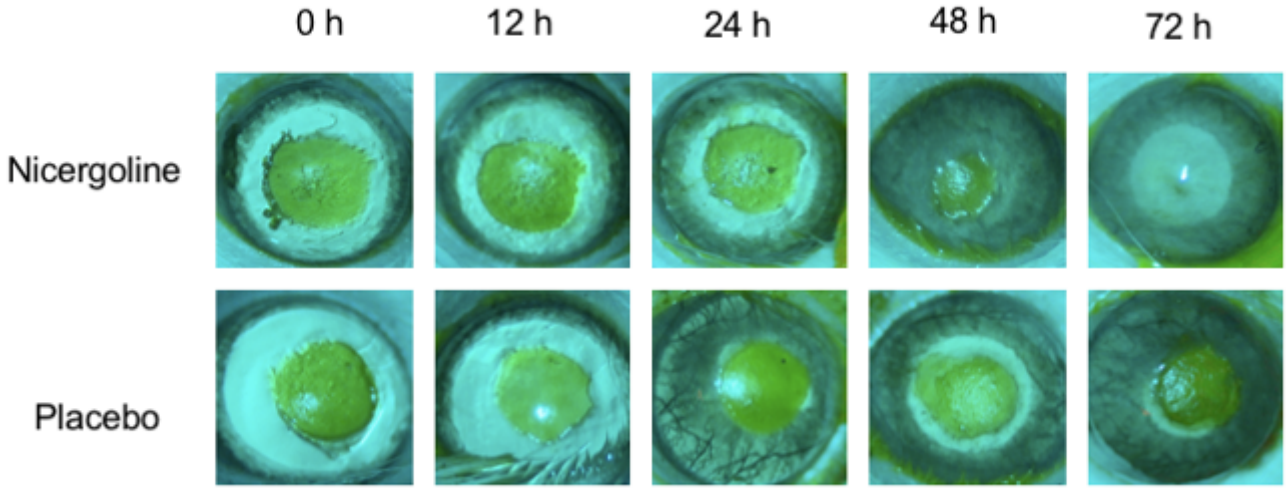
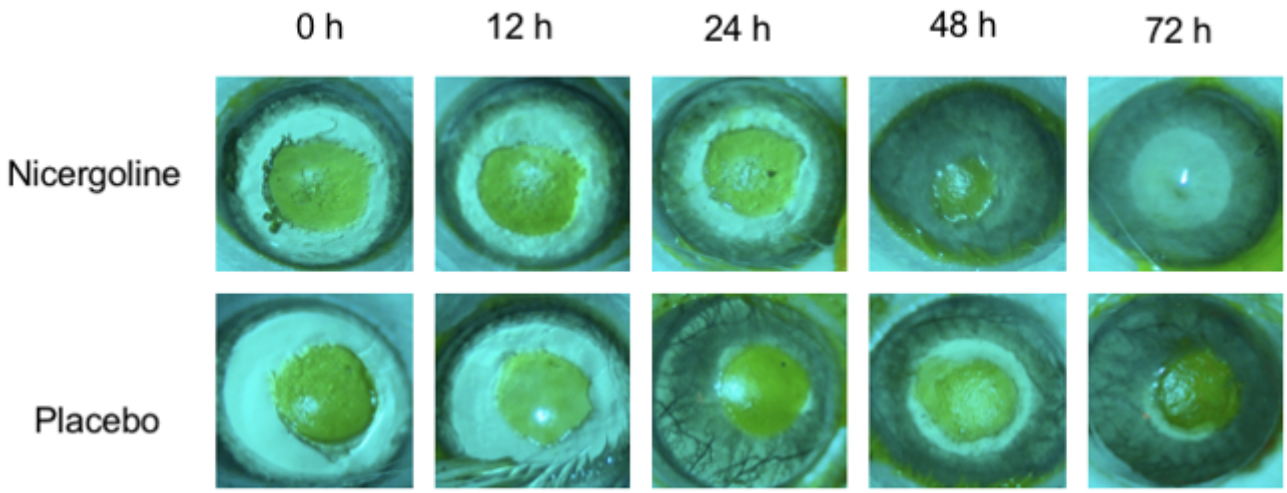


Figure 2

Images of fluorescein corneal staining showing the area of epithelial defect in green along the time interval considered in eyes treated with nicergoline or placebo.



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