

Corneal Epithelial Barrier Function After Oxybuprocaine Provocation in Diabetics

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Corneal epithelial permeability for fluorescein was determined after provocation by a local anesthetic in 18 non-insulin-dependent diabetes mellitus (NIDDM) patients, 23 insulin-dependent diabetes mellitus (IDDM) patients, and 22 healthy controls to evaluate the corneal epithelial barrier function in diabetes. All volunteers had Oxybuprocaine instilled into one eye and saline into the other eye. The epithelial permeability values were determined by fluorophotometry, and the ratio between both eyes was calculated for each individual. The mean permeability values of the saline-instilled eyes in the diabetic patients did not differ significantly from those in the healthy controls ($P > 0.2$). The individual ratios between Oxybuprocaine- and saline-instilled eyes in the NIDDM and IDDM patients differed significantly from those in the healthy controls (mean ratios: 2.6, 1.9, and 1.0, respectively; $P < 0.002$). The permeability ratios and the percentage glycosylated hemoglobin (HbA1c) were linearly correlated in the NIDDM patients but not in the IDDM patients ($r = 0.73$, $P < 0.001$, and $r = 0.09$, $P > 0.68$, respectively). The results showed that the corneal epithelial barrier function in the diabetic patients was not impaired compared with that in the healthy controls. After provocation by a local anesthetic, the barrier function was impaired in the diabetic patients only. *Invest Ophthalmol Vis Sci* 31:436–439, 1990

Diabetic patients more often show corneal epithelial abnormalities than do healthy individuals. These abnormalities may involve recurrent corneal ulcerations^{1,2} or a decrease in epithelial damage threshold and corneal sensitivity.^{3,4} Furthermore, local anesthetics and preservatives can exert a toxic effect on the corneal epithelium in healthy individuals, resulting in an increased permeability that can be detected by fluorophotometry, even before changes can be seen by slit-lamp examination.^{5,6}

To our knowledge only one functional study on the corneal epithelium of diabetic patients by means of fluorophotometry has been published.⁷ The aim of our study was to determine the corneal epithelial barrier function in diabetic patients by using a simple and reliable method⁸ and to assess the effect of instilling a local anesthetic into the eye.

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Materials and Methods

Non-insulin-dependent diabetic patients (NIDDM), insulin-dependent diabetic patients (IDDM), and healthy controls participated in this study (Table 1). The mean age did not differ significantly among the groups. The diabetic patients were selected from the Outpatient Department of the Leiden University Hospital Eye Clinic (Leiden, the Netherlands) and the healthy controls by advertisements in local newspapers. Individuals were selected on the basis of an assessment by slit-lamp examination that all corneal layers of both eyes were normal. Individuals with 1) a history of corneal eye disease, 2) eye medication, 3) contact lenses, or 4) photocoagulation treatment in the past 3 months were excluded from the study.

The study was approved by the Medical Ethical Committee of the Leiden University Hospital, and informed consent was obtained from each individual after the nature of the procedure had been explained fully.

Fluorophotometric measurements were carried out with the Fluorotron Master (Coherent Radiation, Palo Alto, CA) according to a method described previously.⁸ An esthesiometer from Cochet and Bonnet (Luneau, France) was used for the determination of the corneal sensitivity. An 0.4% Oxybuprocaine HCl

Table 1. Age and diabetes duration

Group	Number of individuals	Age (yr)		Diabetes duration (yr)	
		Mean \pm SD	Range	Mean \pm SD	Range
NIDDM	18	58.0 \pm 17.3	32-81	9.2 \pm 6.8	1-28
IDDM	23	48.3 \pm 16.8	24-79	17.5 \pm 7.5	5-30
Control	22	49.3 \pm 21.6	18-80	—	—

solution (USP XXI) containing 0.01% benzalkonium chloride (Ph Eur) as a preservative, boric acid (Ph Eur), and edetate disodium salt (USP XXI) was used as a local anesthetic. Saline was used as a placebo. (Both solutions were obtained from the hospital pharmacy.)

Experimental Protocol

First, a blood sample was taken from the diabetic patients to determine the momentary percentage glycosylated hemoglobin (HbA1c).

After esthesiometry⁴ and corneal slit-lamp examination without the use of fluorescein, four fluorophotometric scans of each cornea were carried out in each individual to determine the mean peak value of the corneal autofluorescence.⁸ Then one drop of Oxybuprocaine was instilled into the conjunctival sac of one eye, and one drop of saline into the other eye. This procedure was repeated after 2 min. Two minutes after the last instillation, eye baths containing 1% fluorescein in saline were applied simultaneously to both eyes for 3 min. Afterwards, both eyes were rinsed for 5 min by means of eye baths containing saline. The left and the right eye then were scanned in turn every 2 min for 1 hr (each eye 15 times).

Finally, slit lamp examination was performed after fluorescein staining of the cornea for the detection of minimal corneal damage.

For each eye, the corneal epithelial permeability value for fluorescein was determined from the fluorescein concentration in the cornea as measured by fluorophotometry,⁸ and the individual ratio between the values of the Oxybuprocaine- and saline-instilled eye was calculated. The mean of the temporal, nasal,

inferior, and superior esthesiometry values of both corneas was calculated.

Results

The mean corneal epithelial permeability values of Oxybuprocaine- and saline-instilled eyes and the means of the ratios between permeability values of both eyes in NIDDM, IDDM patients, and healthy controls are presented in Table 2. The mean ratio in each patient group differed significantly from the ratio in the healthy controls ($P < 0.002$), but no significant difference was found between the two patient groups ($P > 0.1$). The mean permeability value of the saline-instilled eyes in the NIDDM and IDDM patients did not differ significantly from that in the healthy controls ($P > 0.2$ and $P > 0.6$, respectively).

The mean corneal sensitivity values in the NIDDM and IDDM patients differed significantly from that in the healthy controls (mean = 56.1 mm \pm 6.2 SD, 56.9 mm \pm 5.7 SD, and 59.8 mm \pm 0.06 SD, respectively; $P < 0.05$).

The permeability ratios of the NIDDM, IDDM, and healthy controls were independent of age (linear correlation coefficients: $r = -0.16$, $r = 0.24$, and $r = -0.14$, respectively; Fig. 1). The ratios of the diabetic patients correlated neither with the diabetes duration ($r = -0.20$) nor with the corneal sensitivity ($r = -0.005$). The permeability ratios correlated with the HbA1c values in the NIDDM patients but not in the IDDM patients ($r = 0.73$, $P < 0.001$, and $r = 0.1$, $P > 0.68$, respectively; Fig. 2).

Superficial punctate keratopathy was seen on slit-lamp examination in the Oxybuprocaine-instilled eyes in seven NIDDM patients (38.9%), seven IDDM

Table 2. Corneal epithelial permeability after instillation of oxybuprocaine or saline

Group	Number of individuals	Mean permeability \pm SD (nm/sec)		Mean ratio* \pm SD	P†
		Oxybuprocaine	Saline		
NIDDM	18	0.087 \pm 0.051	0.043 \pm 0.023	2.6 \pm 1.7	<0.002
IDDM	23	0.062 \pm 0.048	0.033 \pm 0.020	1.9 \pm 1.2	<0.002
Control	22	0.034 \pm 0.019	0.036 \pm 0.016	1.0 \pm 0.5	—

* Mean of the individual ratios between the permeability values of Oxybuprocaine- and saline-instilled eyes.

† P value of two samples (two-tailed) student t -test between the mean ratio of the patient group and that of the control group.

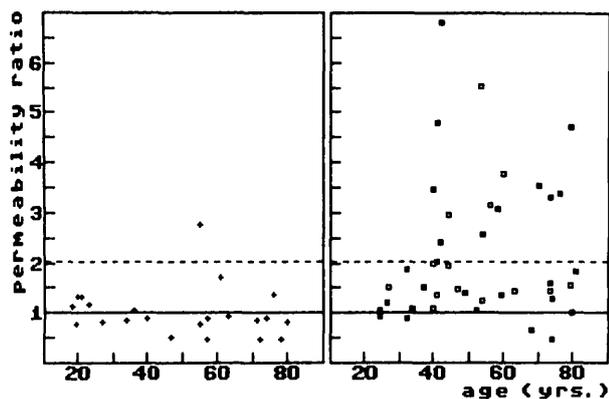


Fig. 1. Ratio between corneal epithelial permeability values in Oxybuprocaine- and saline-instilled eyes, versus age. The solid line represents a ratio of 1, and the dashed line the 95% probability interval for healthy controls. (Left) +, healthy controls. (Right) solid squares, NIDDM patients; open squares, IDDM patients.

patients (30.4%), and two healthy controls (9.1%). No superficial punctate keratopathy was seen in the saline-instilled eyes.

Discussion

The results indicated that corneal provocation by instillation of two drops of Oxybuprocaine with benzalkonium chloride as a preservative increased the corneal epithelial permeability for fluorescein significantly in the diabetic patients but not in the healthy controls.

The corneal epithelial barrier function was not impaired in the saline-instilled eyes of the diabetic patients and in the Oxybuprocaine- and saline-instilled eyes of the healthy controls. The corresponding permeability values did not differ significantly from those of noninstilled eyes in healthy controls in a previous study⁸ ($n = 86$, mean $0.038 \text{ nm} \pm 0.017 \text{ SD}$, $P > 0.2$). It should be noted that in this previous study the permeability values were found independent of age,⁸ and that in our study the three groups did not differ with respect to age. It has been shown by scanning electron microscopy that topical anesthetics can induce minimal morphologic changes in the corneal epithelium.⁵ However, in a later *in vivo* study, an impairment of the epithelial barrier function did occur after at least three drops of local anesthetic.⁶ This suggests that by using two drops in healthy controls, the possible morphologic changes do not affect the barrier function of the epithelium.

The results of our study contrast those of a recent study which showed that the corneal epithelial barrier function in diabetic patients was impaired compared to that of healthy individuals.⁷ In that study, the relative corneal epithelial permeability was determined from the corneal fluorescence measured 45 min after

instillation of $40 \mu\text{l}$ fluorescein. Compared to the bathing method used in our study,⁸ this instillation method has serious drawbacks: 1) Due to interindividual differences in thickness of basal and reflex tear film and the limited spatial resolution of the Fluorotron Master, the calculated fluorescein concentration must be corrected with a factor varying from 200 to 1000, depending on the tear film thickness; 2) reflex lacrimation is caused by instillation of $40 \mu\text{l}$ fluorescein; 3) the amount of reflex and basal lacrimation differs among individuals,⁹ and 4) a major portion of the $40 \mu\text{l}$ of fluorescein is lost in an unpredictable way, since tear volume amounts to about $7 \mu\text{l}$. The last three drawbacks listed result in various and complex decays of fluorescein in the tear film, and thus, varying amounts of fluorescein diffuse into the corneal stroma.

Superficial punctate keratopathy was seen more frequently in the NIDDM and IDDM patients than in the healthy controls (38.9%, 30.4%, and 9.1%, respectively). This confirms a previous study which reports an increased susceptibility to corneal epithelial damage in diabetic patients.¹

In accordance with a previous study,⁴ the corneal sensitivity of the diabetic patients was significantly decreased in comparison with that of the healthy controls. It has been suggested that a decrease in sensory innervation of the cornea can induce a decrease in the corneal epithelial function.¹⁰ Our results do not confirm this suggestion, since in the diabetic patients no significant correlation was found between the permeability ratios and the corneal sensitivity. Apparently, the sensory innervation of the cornea of the

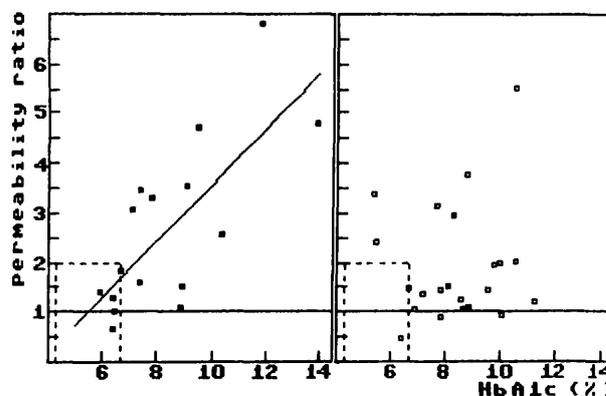


Fig. 2. Ratio between corneal epithelial permeability values in Oxybuprocaine- and saline-instilled eyes, versus percentage HbA1c in diabetic patients. The line sloping upwards was obtained by linear regression analysis of the data points; the vertical dashed lines indicate the HbA1c range for healthy individuals. The solid horizontal line represents a ratio of 1, and the dashed horizontal line represents the 95% probability interval for healthy controls. (Left) NIDDM patients (solid squares). (Right) IDDM patients (open squares).

diabetic patients was insufficiently impaired to result in a decrease of the epithelial barrier function.

Long-term diabetes did not seem to affect the corneal epithelial function, since in both diabetic patient groups no correlation was found between the permeability ratios and the diabetes duration. The correlation between the permeability ratios and the percentage HbA_{1c}, found in the NIDDM patients but not in the IDDM patients, indicated that a good metabolic regulation of diabetes affected the corneal epithelial function in the NIDDM patients only. Currently, we are unable to explain this discrepancy.

The morphologic equivalent of the corneal epithelial barrier function is the extent to which the epithelial intercellular clefts are closed by zonulae occludentes, or tight junctions. Diabetic patients can show elevated corneal sorbitol contents and corneal basement membrane abnormalities.² These conditions lead to osmotic changes in the cornea that may result in stromal and epithelial edema.¹¹ Edema can stretch the zonulae occludentes, thereby affecting the fusion of the adjacent cell membranes within the epithelium. These changes can increase the susceptibility to an impairment of the epithelial barrier function after provocation with oxybuprocaine, since this local anesthetic has a toxic effect on the epithelial cell membrane.⁵

It is known that ophthalmic solutions other than 0.4% oxybuprocaine can exert similar effects on the corneal epithelium⁶ and that diabetic patients may show a delayed epithelialization.¹ Therefore, we suggest that in diabetic patients, the number of instillations of local anesthetics containing benzalkonium chloride as a preservative should be restricted.

Key words: corneal epithelial permeability, fluorophotometry, local anesthesia, diabetes mellitus, glycosylated hemoglobin

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