Long term changes in human corneal endothelium following toxic endothelial cell destruction: a specular microscopic and fluorophotometric study

Rudy M M A Nuyts, Nicolien Boot, Jaap A van Best, Henry F Edelhauser, Adrian C Breebaart

Abstract

Aims—To investigate the long term relation between corneal thickness, endothelial morphometric variables, and endothelial permeability in patients with endothelial cell counts under 900 cells/mm² as a result of endothelial cell destruction after cataract surgery.

Methods—Eighteen patients developed the so called toxic endothelial cell destruction (TECD) syndrome following routine cataract surgery because of the intra-cameral injection of a toxic detergent residue. Ten patients with a mean (SEM) initial cell loss of 72% (2%) were followed for 4 years. Data were obtained at 6 months and 4 years postoperatively and compared between TECD eyes and contralateral control eyes.

Results—Mean (SEM) endothelial cell density of the TECD eyes increased from 642 (41) cells/mm² to 849 (50) cells/mm² at 4 years postoperatively ($p=0.005$). There was no difference in coefficient of variation or percentage hexagonals between 6 months and 4 years postoperatively. Mean (SD) corneal thickness of the TECD eyes and control eyes was similar, 0.51 (0.02) mm and 0.49 (0.01) mm, respectively ($p=0.65$). Mean (SD) endothelial permeability was also similar for TECD eyes and control eyes ($4.3 (0.9) \times 10^{-4}$ cm/min and $4.4 (0.6) \times 10^{-4}$ cm/min, respectively ($p=0.57$). There was no correlation between endothelial cell density, coefficient of variation, or percentage of hexagonal cells and endothelial permeability in the TECD eyes. In three patients a permanent corneal decompensation occurred.

Conclusions—Four years after TECD corneal endothelial wound healing is stable and the barrier function has been restored.

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substance and the detergent was probably combined with the residual viscoelastic material. Endothelial morphometric analysis showed a mean (SEM) endothelial cell loss of 72% (2%), 3 and 6 months postoperatively compared with the contralateral eyes. Endothelial wound healing, as determined by coefficient of variation and percentage hexagonals, was stable 6 months postoperatively.

There are numerous published studies on the morphometric follow up of the corneal endothelium after routine cataract surgery.³⁻¹³ Usually, a cell loss of 10 to 20% is reported, dependent on the duration of follow up. Few reports exist on the long term follow up of patients who developed severe endothelial cell loss at the time of surgery.¹⁴⁻¹⁶ It is thought that after severe cell loss, as is described in our series, there will be increased cell loss of the corneal endothelium, similar to that attributed to the aging effect.¹⁵⁻¹⁷ Consequently, permanent decompensation of these compromised corneas may occur. It is also possible that a low endothelial cell density and an unstable monolayer may decrease the barrier function of the corneal endothelium.

The purpose of this study was to investigate the long term relation between corneal thickness, endothelial morphometric variables, and endothelial permeability in a series of 10 patients with initial endothelial cell counts under 900 cells/mm².

Materials and methods

MORPHOMETRIC ANALYSIS

Non-contact specular microscopy of the central cornea (Nikon camera, Tokyo, Japan)

Figure 1 Case 9. Corneal decompensation 1 month after extracapsular cataract extraction. Note the extensive corneal clouding.

In 1990 we described a series of patients who developed an acute corneal decompensation following routine cataract extraction in 1988, characterised by star-shaped endothelial folds and a twofold increase in corneal thickness (Fig 1).¹ This toxic endothelial cell destruction (TECD) syndrome was caused by the intra-cameral injection of a toxic detergent residue inside reusable irrigating cannulas.² The cannula was previously used to inject a viscoelastic

Department of
Ophthalmology,
University of
Maastricht, the
Netherlands
R M M A Nuyts

Department of
Ophthalmology,
Academic Hospital,
University of Leiden
N Boot
J A van Best

Department of
Ophthalmology,
Emory University,
Atlanta, Georgia
H F Edelhauser

Department of
Ophthalmology,
Academic Medical
Center, University of
Amsterdam, the
Netherlands
A C Breebaart

Correspondence to:
R M M A Nuyts,
Department of
Ophthalmology, University
of Maastricht, P Debye-
laan 25, Postbus 5800, 6202 AZ
Maastricht, the Netherlands.

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of the initially decompensated and the unaffected contralateral eyes was performed at 6 months after intraocular surgery. Of the 18 patients with TECD three patients died, two underwent keratoplasty, one left the country, and two patients refused participation in the study. Specular photographs of the remaining 10 patients were used for morphometric analysis 4 years postoperatively. The age of these subjects ranged from 32 to 85 years (mean 66.8 years). The endothelial photographs were analysed using 100 cells per photograph as previously described for morphological analysis.¹⁸ Briefly, endothelial photographs of the central corneal endothelium were selected randomly and analysed in a masked fashion by the same technician using 100 neighbouring cells from one specular micrograph. Cell morphological variables studied included endothelial cell size (μm^2), endothelial cell density (ECD, number of cells/ mm^2), coefficient of variation in cell size, and percentage of hexagonal cells (%). We compared the values of the eyes with the TECD syndrome with the contralateral eyes using the Wilcoxon signed rank test. In addition, we performed a repeated measures analysis of variance to determine changes in mean cell area (SD) between 6 months and 4 years postoperatively.

ENDOTHELIAL PERMEABILITY MEASUREMENTS

Patients 1 and 6 refused participation in the study because of general health problems precluding the intensive examination of the experiment itself. Three participating patients had systemic disease including one patient with coronary heart disease (patient no 5), one patient with hypertension and peripheral vascular disease (patient no 8), and one with diabetic mellitus (patient no 10). Systemic medication included insulin (patient no 10), anticoagulation drugs (patients 8 and 5), diuretics (patient no 8), anti-anxiety drugs (patient no 5), and H_2 receptor blocking agents (patient no 7). Any local eye medication was interrupted 2 days before measurement of the endothelial permeability, except for patient no 10, who continued timolol maleate 0.5% eyedrops once daily in both eyes. Previous ocular surgery and ocular abnormalities of the contralateral eye were registered. Informed consent was obtained from each patient after the nature of the procedure was fully explained. Slit-lamp biomicroscopy and ophthalmoscopy of the eyes were performed 2 weeks before measurement of the endothelial permeability. Both corneas were examined to assess signs of epithelial lesions, corneal oedema, or guttata. The thickness of the cornea was measured by optical pachymetry with the Haag-Streit slit-lamp. On the day of fluorophotometry, four drops of a 10% fluorescein solution were instilled in the conjunctival sac of both eyes with an interval of 2–4 minutes. Excess fluorescein was removed from the eyelids with moistened tissues. The fluorescein concentration in the cornea and anterior chamber was measured four times at 4, 5, 6, and 7 hours after instillation of the drops

using the Fluorotron Master (Coherent Radiation Inc, Palo Alto, CA, USA) equipped with an anterior segment adaptor.¹⁹ The patient was asked to blink just before the start of the fluorophotometry scan, and to suppress the blinking reflex during the experiment. At the end of the measurements slit-lamp examination of both eyes was performed to check the corneas for any irregularities.

CALCULATIONS

Quantitative measurements of endothelial permeability were performed according to the methods and calculations as described by Van Best *et al* in a large European multicentre study using a standardised protocol.¹⁹ For determination of endothelial permeability the following expression was used:

$$P = d_{\text{cm}} \cdot \alpha_c \cdot \frac{C_c}{r_{\text{ac}} \cdot C_c - C_a} \quad (\text{m/s})$$

with P is endothelial permeability, d_{cm} is the corneal thickness, α_c is a decay constant for the fluorescein concentration in the cornea approximated by a single exponential curve, C_c and C_a are the fluorescein concentrations in the cornea and anterior chamber (kg/m^3) and r_{ac} is the unbound part of corneal fluorescein. Fluorophotometry measurements in the cornea and anterior chamber were corrected for autofluorescence and the fluorescein concentration in the cornea for limited spatial resolution with a factor 1.57. The r_{ac} value corresponds to the steady state ratio between anterior chamber and cornea and a value of 0.64 was used. The values for corneal and anterior chamber autofluorescence were 13.5 ng Eq/ml and 2.5 ng Eq/ml, respectively. The corneal and anterior chamber fluorescence values $C_c(t_m)$ and $C_a(t_m)$ are determined for $t_m = 240, 300, 360$, and 420 minutes and corrected for autofluorescence.

Comparisons and correlations between measured values were performed and analysed using the Mann-Whitney U test and the non-parametric Spearman correlation test.

Results

Table 1 summarises the morphometric analysis performed on the 10 patients. Six months postoperatively the mean (SEM) of the endothelial cell density was 642 (43.4) cells/ mm^2 and 2345 (184.4) cells/ mm^2 for the TECD eyes and contralateral eyes, respectively. The lowest cell count was 463 cells/ mm^2 (case no 10) and the highest cell count 898 cells/ mm^2 (case no 5). The mean (SEM) percentage cell loss, compared with the contralateral eyes, at 6 months was 72% (2%). At 4 years postoperatively, endothelial cell density was 849 (52.1) cells/ mm^2 and 2128 (165.0) cells/ mm^2 for the TECD eyes and contralateral eyes, respectively. The lowest cell count was 625 cells/ mm^2 (case no 8) and the highest cell count 1103 cells/ mm^2 (case no 9). The average percentage cell loss at 4 years was 60% (2%), compared with the contralateral eyes. There

Table 1 Specular microscopy and permeability data after toxic endothelial cell destruction syndrome

No, age, sex	Cell area OE* (μm^2)		Cell area CE* (μm^2)		Percentage ECD** difference OE vs CE		Endothelial permeability (10^{-4} cm/min)		Corneal thickness (mm)		Ocular history (CE)
	6 Months	4 Years	6 Months	4 Years	6 Months	4 Years	OE	CE	OE	CE	
1, 70, F	2059 (519)	1313 (346)	347 (113)	762 (203)	83	42	—	—	—	—	ECCE+PCL†, POAG‡
2, 60, F	1836 (523)	1088 (260)	676 (175)	644 (182)	63	41	6.70	4.10	0.58	0.52	ECCE+PCL
3, 59, F	1442 (469)	1160 (343)	339 (114)	371 (121)	77	68	3.00	3.60	0.54	0.54	PACG§
4, 85, M	1759 (573)	1460 (416)	527 (186)	488 (190)	70	67	1.20	2.80	0.48	0.48	POAG
5, 75, F	1116 (373)	916 (234)	386 (118)	385 (105)	65	58	3.90	4.40	0.47	0.49	ECCE+PCL
6, 80, F	1457 (335)	1118 (405)	595 (168)	581 (181)	59	48	—	—	—	—	None
7, 80, M	1557 (793)	1157 (271)	467 (126)	346 (92)	80	70	3.90	4.20	0.60	0.54	ECCE+PCL
8, 76, F	1225 (260)	1600 (611)	430 (121)	542 (140)	65	66	9.50	6.40	0.50	0.47	ECCE+PCL
9, 32, F	2036 (677)	907 (415)	386 (96)	418 (97)	77	54	2.30	7.50	0.50	0.50	Panuveitis
10, 51, M	2158 (1159)	1468 (391)	532 (385)	450 (337)	75	69	3.90	2.50	0.44	0.44	ECCE+ACL¶

*Mean (SD) cell area in operated eye (OE) and control eye (CE); **percentage of endothelial cell density (ECD) difference between the operated eye and contralateral eye; †extracapsular cataract extraction with posterior chamber lens implant; ‡primary open angle glaucoma; §primary angle closure glaucoma; ¶extracapsular cataract extraction with anterior chamber lens implant.

was an increase in the mean endothelial cell density of the TECD eyes between 6 months and 4 years postoperatively ($p=0.005$, Wilcoxon signed rank test). There was a significant decrease in cell area of TECD eyes between 6 months and 4 years postoperatively ($p<0.001$, Bonferroni repeated measures analysis of variance). There was no difference in cell area for the contralateral eyes or for the standard deviations of the individual cell areas of both TECD eyes and contralateral eyes between 6 months and 4 years postoperatively ($p>0.05$, Bonferroni repeated measures analysis of variance). One eye of the TECD eyes had epithelial and stromal oedema (patient no 2) and the TECD eye of patient no 7 had corneal guttata without oedema. Patient no 8 had severe corneal guttata and endothelial oedema in both eyes. Patient no 4 had mild corneal guttata in the control eye. Figure 2 shows the endothelial photographs of case no 3 during the 4 year follow up period. The endothelial micrograph 4 years after endothelial cell destruction shows a restored monolayer.

There was no significant difference in endothelial coefficient of variation between TECD eyes and contralateral eyes at any time (Table 2). At 6 months postoperatively but not at 4 years postoperatively, there was a significant difference in the percentages of hexagonal cells between TECD eyes and the contralateral eyes (Table 2, $p=0.01$ and $p=0.19$, respectively). There was no significant change in percentages of hexagonal cells for TECD eyes or contralateral eyes from 6 months to 4 years postoperatively ($p=0.85$ and $p=0.08$, respectively). The mean endothelial permeability values of the TECD eyes and of the control eyes were similar, $4.3 (0.9) \times 10^{-4}$ cm/min and $4.4 (0.6) \times 10^{-4}$ cm/min, respectively (Table 2, $p=0.57$). There was no significant difference in endothelial permeability between TECD eyes and the control eyes of five patients who had

undergone an extracapsular cataract extraction in the control eye, $5.6 (1.1) \times 10^{-4}$ cm/min versus $4.3 (0.6) \times 10^{-4}$ cm/min, respectively ($p=1.0$). No correlation was found between endothelial cell density, coefficient of variation, or percentage of hexagonal cells and endothelial permeability in the TECD eyes ($p=0.50$, $p=0.43$, and $p=0.88$, respectively; non-parametric Spearman correlation test).

Mean corneal thickness of the eyes with TECD and those of the control eyes was similar, $0.51 (0.02)$ mm and $0.49 (0.01)$ mm, respectively ($p=0.65$). There was no correlation between the corneal thickness and the endothelial permeability in the TECD eyes or the control eyes ($p=0.65$ and $p=0.50$, non-parametric Spearman correlation test).

Discussion

This study represents the first series of patients with long term follow up of endothelial morphometry and fluorophotometry after severe endothelial cell loss due to toxic endothelial cell destruction. We believe that the clinical picture of this particular group of patients differs from the usual postcataract patient group with respect to endothelial wound healing characteristics. After routine cataract surgery the greatest cell loss is usually in the superior cornea, since the incision is made superiorly and the lens extraction is most traumatic to the superior cornea.⁴ However, in the TECD group extensive cell loss occurred in the central cornea, as a result of the circulation of the toxic substance in the anterior eye chamber. Therefore the great cell loss centrally may lead to a spreading and migration response of the peripheral corneal endothelium towards the central cornea. Another explanation for the increase in central endothelial cell density from 649 to 839 cells/mm² after 4 years could be that the cell density increased through cell division. Although the mitotic activity of the human

Table 2 Summary of specular microscopy and endothelial permeability data (mean (SEM))

Follow up	Endothelial cell density (cells/mm ²)		Coefficient of variation		Percentage hexagonals (%)		Endothelial permeability (10^{-4} cm/min)	
	TECD	Control	TECD	Control	TECD	Control	TECD	Control
6 Months	642 (43.6)	2345 (184.4)	0.33 (0.03)	0.34 (0.04)	53 (4.0)	64 (2.7)*	—	—
4 Years	849 (52.1)*	2128 (165.0)	0.30 (0.02)	0.34 (0.05)	55 (2.0)	60 (2.7)	4.3 (0.9)	4.4 (0.6)

* $p=0.005$ compared with TECD eye at 6 months.

Figure 2 Case 3. (A) Endothelial micrograph of the eye with toxic endothelial cell destruction (TECD) 6 months after cataract surgery. Note the endothelial oedema and large cells. The endothelial cell density is 694 cells/mm². (B) Endothelial cell layer of the TECD eye 2 years after cataract surgery shows a regular endothelial cell layer with an endothelial cell density of 919 cells/mm². (C) Four years after surgery the endothelial monolayer is still intact. No endothelial oedema is seen and the endothelial cell density is 861 cells/mm². (D) The unoperated contralateral eye shows a regular endothelial monolayer with an endothelial cell density of 2699 cells/mm².

Fig 2A

Fig 2B

Fig 2C

Fig 2D

corneal endothelium is very limited, some mitosis of the endothelium has been described in vitro and in vivo.²⁰⁻²² In cats, also a species with very limited replication of corneal endothelial cells, centrally induced mechanical trauma also led to a slight increase in endothelial cell density after 18 months.²³ In addition to the aforementioned hypotheses for the increase in endothelial cell density, it is known that the accuracy of endothelial cell density measurements sometimes can be questioned and considerable sample errors may occur.²⁴ However, the stability of the mean endothelial cell area in the control eyes and the lack of a significant difference between standard deviations of cell area for both TECD eyes and control eyes suggest an accurate method of specular microscopy and endothelial morphometry.

There are studies that have established the factors of endothelial function after cataract surgery used to predict future endothelial decompensation.¹⁴⁻¹⁶ It is believed that large endothelial cell loss in the early postoperative phase is a risk factor for future endothelial decompensation.^{15,16} Hoffer described a case report of a patient with a cell count of 370 cells/mm², who developed a bullous keratopathy 1 year after this measurement.¹⁴ In our series with morphometry data only one patient, patient no 2, developed a corneal decompensation 9 months after the 4 year follow up specular photography and fluorophotometry

measurements. At that time, the endothelial cell density was 919 cells/mm², much higher than the patient reported by Hoffer. Unfortunately, the two other patients in this group who needed a corneal transplantation never recovered clear corneas after the initial decompensation that permitted specular microscopy.

Bates emphasised the importance of the first postoperative month cell loss as a predictor for bullous keratopathy with long term follow up.^{15,16} In a longitudinal study on endothelial morphometry after cataract surgery he found a mean endothelial cell density of 1726 (647) cells/mm² 6 months after surgery, in a group of patients who ultimately would develop a bullous keratopathy. The mean time period for corneal decompensation after cataract surgery was 3.5 years postoperatively and the mean endothelial cell density at this point was 515 (97) cells/mm². Obviously, despite even a lower mean cell density 6 months postoperatively, cell loss in the TECD group does not progress in the same rate as reported by Bates. In Bates's series factors like low grade uveitis, vitreous loss during cataract surgery, or intraocular lens malposition may have contributed to a continuous, possibly immune mediated, damaging effect on the endothelial monolayer.

Other authors suggest that the preoperative coefficient of variation and percentage of

hexagonal cells are related to the risk for the development of postoperative corneal oedema and susceptibility to surgical trauma.²⁵ This point has been confirmed in corneal endothelial wound healing studies of cats, where a correlation was found between permanent endothelial decompensation and a decrease of endothelial cell density to 40% of baseline values, a decrease in hexagonals to 33%, and a three to fourfold increase in coefficient of variation, respectively.²⁶ In addition, a correlation also was shown between endothelial dysfunction and the occurrence of giant endothelial cells with a surface area 7.5–13.5 times of normal endothelial cells.²⁶ Despite our high initial cell loss of 72% we were unable to demonstrate a similar relation. Furthermore, no statistical difference was found for either the coefficient of variation or the percentage of hexagonal cells between 6 months and 4 years postoperatively suggesting that a stable endothelial layer can be maintained for more than 4 years, even after severe cytotoxicity trauma to the corneal endothelium.

Our study showed no correlation between endothelial permeability and the endothelial variables of wound healing 4 years after severe endothelial trauma. The corneal endothelial permeability of the TECD eyes was within the range of normal values as reported by various investigators.^{19–27–29} These results are in accordance with earlier reports that showed a return of endothelial permeability to normal values 3 months after routine cataract surgery.³⁰ Similarly, endothelial permeability returned to normal values 3 months after traumatic wounding of cat endothelium.³¹ However, other studies have shown increased endothelial permeability values within 3 months after intraocular surgery.³² One study in patients after keratoplasty showed a decreased endothelial permeability, possibly owing to the decrease in intercellular space which could be the result of the increased incidence of giant endothelial cells.³³ Despite the prevalence of many large endothelial cells in our series we were unable to confirm these findings. Based on our results it appears that the endothelial permeability, even after extensive endothelial cell destruction, does not increase as long as a restored monolayer is present. However, in cases with a disruption or a stressed state of the monolayer, as was seen in the patients with corneal oedema or guttata, increased permeability values were determined.

After severe endothelial cell loss a delicate balance between corneal oedema and clarity exists. At present, we do not know to what extent the endothelial pump should increase its activity in corneas with low cell counts to maintain corneal clarity. Probably, different stress factors to the corneal endothelium should be discerned: an acute toxicity mechanism with extensive endothelial cell loss, followed by a quick response in recovery of corneal clarity and stabilisation of the monolayer versus a gradual cell loss associated with the presence of a continuous inflammatory

stimulus. These additional stimuli may exceed the capacity of the endothelial pump and/or the endothelial barrier in a compromised endothelium to maintain corneal clarity. Previous studies support this hypothesis since intraocular inflammation has been shown to affect corneal oedema by both an increase in endothelial permeability and a decrease in Na/K ATPase pump site density.³⁴

In summary, the results of this study suggest that the human corneal endothelium can recover from toxic endothelial cell destruction provided there are enough endothelial cells to migrate over Descemet's membrane and re-establish the barrier. In this series of patients, the majority recovered; however there were three patients where the endothelial cell destruction exceeded the ability of the endothelial cells to recover, and the cornea decompensated.

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