



The Effect of Fluorescein on Corneal Endothelial Structure and Morphology in Diabetic Retinopathy Patients undergone Fundus Fluorescein Angiography

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Abstract

Objectives: To evaluate fluorescein effect on the corneal endothelium by endothelial specular microscopy in diabetic retinopathy patients undergone fundus fluorescein angiography (FFA).

Methods: One hundred thirty four diabetic retinopathy patients undergone FFA aged between 42 and 74 years were included study. We checked the corneal endothelial cell density (ECD), coefficient of variation of cell area (CV/polymegethism) and percentage of hexagonal cells (6A/pleomorphysm) before FFA and 1 hour, 1 day, 1 week after the procedure.

Results: The mean baseline ECD was 2223 ± 216 cells/mm². The mean ECD before and 1 hour (2234 ± 224 cells/mm²), 1 day (2237 ± 231 cells/mm²), 1 week (2226 ± 219 cells/mm²) after the procedure weren't significantly different ($p=0.243$; repeated measure ANOVA). The mean of the coefficient variations of the endothelial cell size before and 1 hour, 1 day, 1 week after the procedure were 0.327 ± 0.07 , 0.319 ± 0.06 , 0.322 ± 0.06 , 0.325 ± 0.07 respectively ($p=0.281$; repeated measure ANOVA). The mean of the baseline percent of hexagonal cells (pleomorphism) in the endothelium was 61.7 ± 5.2 , and the postoperative 1 hour, 1 day, 1 week percent were 62.3 ± 5.7 , 61.9 ± 6.1 , 61.3 ± 5.9 , respectively ($p=0.317$; repeated measure ANOVA).

Conclusion: The results of this study demonstrated that fluorescein has no toxic effect on corneal endothelium.

Keywords: Corneal endothelial cell density, fluorescein, pleomorphism, polymegethism, toxicity

Introduction

In normal subjects and in diabetics with mild retinopathy, anterior chamber the fluorescein time course was described by Goldmann (1) and Kinsey and Palm (2). The maximal anterior chamber fluorescein concentration was reached after 30–90 minutes and this was followed by a monoexponential decay, while a maximal vitreous fluorescein concentration

was reached two to five hours after intravenous administration and then declined monoexponentially (3).

The rate constant of penetration of fluorescein into the anterior chamber is higher than the penetration into the vitreous as the vitreous is surrounded by a tight barrier compared to the anterior chamber (4). The rate constant of fluorescein penetration into the anterior chamber is a composite constant including more than one process of transfer. This

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mainly includes diffusion of fluorescein across the iris vessels directly into the anterior chamber and ciliary secretion into the posterior aqueous followed by flow to the anterior chamber.

The permeability index obtained from the anterior chamber as well as from the vitreous increased with an increasing degree of retinopathy. It was found to be significantly increased in diabetics with background and proliferative retinopathy in both ocular compartments (5). Tracers can also diffuse between the anterior chamber and the corneal stroma. The corneal concentrations increased gradually and exceeded the aqueous concentration after three to four hours in humans, the maximum concentration being reached after four to five hours (6).

The main purpose of this study was to examine the fluorescein effect on the corneal endothelium by endothelial specular microscopy in diabetic retinopathy patients undergone fundus fluorescein angiography (FFA).

Methods

This prospective, nonrandomized study was performed in the Umraniye Research and Training Hospital and involved 134 non-proliferative diabetic retinopathy patients undergone FFA aged between 42 and 74 years. The purpose of the study was explained to all non-proliferative subjects and informed consent was obtained from each of them before beginning the examination. The study was approved by the local ethics committee and conducted according to the criteria set by the Declaration of Helsinki. The exclusion criteria were intraocular pressure (IOP) ≥ 21 mmHg, corneal opacities, contact lens users, and severe dry eye.

Sodium fluorescein (500 mg/5 ml) was administered in a cubital vein. Central images of corneal endothelium were captured for each eye with a non-contact specular microscope. All measurements were taken by one author (UC) before FFA and 1 hour, 1 day, and 1 week after FFA. We checked the corneal endothelial cell density (ECD), coefficient of variation of cell area (CV/polymegethism), and percentage of hexagonal cells (6A/pleomorphism) before FFA and 1 hour, 1 day, and 1 week after the procedure.

Measurements of corneal ECD and morphology were performed using the non-contact specular mode of Confoscan

4 (Nidek Co., Gamagori, Japan). A non-contact endothelial microscope with a 20x probe and a wider field of view was used for the measurement. The patient's head was positioned similar to a slit-lamp examination, and the patient was instructed to look straight ahead into the built-in fixation targets. Automatic focusing was used to ensure the image of the pupil on the monitor was in clear focus and within the aiming circle visible on the monitor. Three successive images were selected for the analysis. The central or paracentral area was determined by the operator and the automated cell analysis detected the overall density, number of sides, and area of each cell as well as the overall pleomorphism and polymegethism indices. The mean ECD, polymegethism, and pleomorphism of the three images were calculated and recorded for statistical analysis.

All statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY, USA). P values less than 0.05 were considered statistically significant. The Kolmogorov–Smirnov test was used to assess the normal distribution of continuous variables. Baseline and post-procedure 1 hour, 1 day, and 1 week exams plus ECD and CV values were compared using repeated measure ANOVA with the Bonferroni correction used as a post hoc test to reveal which alteration was significant after all of the continuous variables were normally distributed.

Results

The mean baseline ECD was 2223 ± 216 cells/mm². The mean ECD before and 1 hour (2234 ± 224 cells/mm²), 1 day (2237 ± 231 cells/mm²), and 1 week (2226 ± 219 cells/mm²) after the procedure were not significantly different ($p=0.243$; repeated measure ANOVA).

The means of the coefficient variations of the endothelial cell size before and 1 hour, 1 day, and 1 week after the procedure were 0.327 ± 0.07 , 0.319 ± 0.06 , 0.322 ± 0.06 , 0.325 ± 0.07 , respectively ($p=0.281$; repeated measure ANOVA).

The mean of the baseline percentage of hexagonal cells (pleomorphism) in the endothelium was 61.7 ± 5.2 , and the means of the percentages at 1 hour, 1 day, and 1 week post procedure were 62.3 ± 5.7 , 61.9 ± 6.1 , and 61.3 ± 5.9 , respectively ($p=0.317$; repeated measure ANOVA).

All values before and after FFA are summarized in Table I.

Table I. Mean Endothelial cell density, CV of endothelial cell size, Percent of hexagonal cells before and after fundus fluorescein angiography

	Baseline	1 hour	1 day	1 week	p*
Endothelial cell density (cells/mm ²)	2223±216	2234±224	2237±231	2226±219	p=0.243
CV of endothelial cell size (%)	0.327±0.07	0.319±0.06	0.322±0.06	0.325±0.07	p=0.281
Percent of hexagonal cells (%)	61.7±5.2	62.3±5.7	61.9±6.1	61.3±5.9	p=0.317

*Repeated measure ANOVA; CV: coefficient of variation.

Discussion

Corneal endothelial structural and functional changes in diabetic patients have been investigated by various studies (7-14). Some studies showed increased corneal thickness in diabetic patients and persistent corneal edema after cataract extraction and vitrectomy suggesting abnormal function of the corneal endothelium (7-10).

Quantitative measurement of endothelial permeability to fluorescein provides a functional assessment of the endothelial monolayer. Lass et al. (8) found increased endothelial permeability in patients with diabetes mellitus, but this result was not confirmed in some other studies (12,15,16). Larsson et al. (16) found normal endothelial permeability, increased corneal autofluorescence, increased corneal thickness, polymegethism, and pleomorphism in subjects with diabetes mellitus type I compared with age-matched controls. They also found similar measurements in the type II diabetic group, who were older and had had diabetes for a shorter period than the type I group. The values in the type II diabetics, however, did not differ significantly from those of the age-matched control group, which had similar changes, presumably the result of aging. Schultz et al. (11) found structural change in the corneal endothelium of diabetic patients, including a high CV (polymegethism) and a decrease in the percentage of hexagonal cells (polymorphism). In our study, the mean baseline ECD was 2223 ± 216 cells/mm², the mean of the CV of the endothelial cell size was 0.327 ± 0.07 and the mean of the baseline percentage of hexagonal cells in the endothelium was 61.7 ± 5.2 as was compatible with these studies.

It has been also demonstrated that terminal fluorescein elimination from the anterior chamber decreased with an increasing degree of retinopathy, while the permeation of fluorescein into the anterior chamber was the same. This seems to indicate that an increased anterior chamber permeability index in diabetics is caused by a decreased elimination of fluorescein rather than an increased fluorescein leakage. This seems to indicate an ocular restriction of fluorescein elimination from the eye, possibly located in the trabecular meshwork. The results are in accordance with clinical reports indicating increased incidence of glaucoma, including open-angle glaucoma, in diabetics (17).

In the present study, we determined the structural effects of fluorescein on the corneal endothelium by specular microscopy in diabetic retinopathy patients undergone FFA. The mean ECD, coefficient variations of the endothelial cell size and percentage of hexagonal cells were not significantly different between baseline and 1 hour, 1 day, and 1 week after FFA. As far as we know, no study has investigated the effect of fluorescein on corneal endothelial structure and morphol-

ogy. The results of this study have demonstrated that fluorescein has no toxic effect on corneal endothelium.

Disclosures

Ethics Committee Approval: The study protocol was reviewed and approved by the institutional ethics committee of Kartal Lutfi Kırdar Education and Training Hospital (Protocol no: 2017/514/106/4). This study was conducted in accordance with the Declaration of Helsinki.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Involved in design and conduct of the study (UC); preparation and review of the study (UC, YO); data collection (YO); and statistical analysis (GD).

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