Short-term Effect of Dorzolamide Hydrochloride on Central Corneal Thickness in Humans With Cornea Guttata

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Objective: To investigate the short-term effect of dorzolamide hydrochloride, a topical carbonic anhydrase inhibitor, on central corneal thickness in patients with cornea guttata.

Design and Methods: In this randomized, placebo-controlled, double-masked, 3-drug crossover study, 20 patients with cornea guttata (mean endothelial cell count, 1321 cells/mm²) and 8 healthy control subjects (mean endothelial cell count, 2483 cells/mm²) were included. Study medications included 2% dorzolamide hydrochloride (Trusopt 2% eye drops; Merck & Co Inc, Whitehouse Station, NJ), 0.9% saline solution (saline placebo), and a solution identical to the carrier substance of dorzolamide in Trusopt (carrier placebo). The study drugs were applied 4 times per day for 1 day only. Central corneal thickness measurements were performed using partial coherence interferometry on every study day at baseline and after 24 hours of study medication treatment.

Main Outcome Measures: Change in central corneal thickness.

Results: The mean thickening in central corneal thickness within 24 hours in eyes with cornea guttata treated with dorzolamide, saline placebo, and carrier placebo was 12.0 µm (95% confidence interval [CI], 7.0-17.1 µm), 0.6 µm (95% CI, −1.0 to 2.2 µm), and 1.3 µm (95% CI, −0.1 to 2.6 µm), respectively.

Conclusion: Application of dorzolamide for 1 day results in a slight but statistically significant thickening of central corneal thickness in patients with cornea guttata.

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Dorzolamide hydrochloride was the first commercially available topical carbonic anhydrase (CA) inhibitor and has been in widespread use for the treatment of elevated intraocular pressure (IOP) since 1995. Dorzolamide is a potent inhibitor of the cytosolic CA isoenzyme II (CA II), which is found in the nonpigmented epithelium of the ciliary processes, where its inhibition results in a decrease of aqueous humor secretion with subsequent reduction of IOP. The corneal endothelium contains CA II as well as the cytosolic CA I, which play a major role in keeping the cornea relatively dehydrated. Dorzolamide has a high activity against CA II and a low activity against CA I. Because dorzolamide is a major inhibitor of CA II, it has the potential to interfere with the pump function of the corneal endothelium, which could lead to corneal edema.

Before dorzolamide was first approved in 1995 by the US Food and Drug Administration for the treatment of elevated IOP, several studies have investigated the efficacy of dorzolamide in lowering IOP, but also the possible adverse effects of this new topical CA inhibitor. All of these studies showed a significant decrease of IOP when applied 3 times per day or 2 times per day in combination with other topical antiglaucoma drugs. These studies revealed that dorzolamide was well tolerated and that there were no clinically significant changes in ocular or systemic safety parameters. The main outcome measures of the safety parameters investigated in those studies were endothelial cell count (ECC) and corneal thickness (CT) or central CT (CCT). Optical slitlamp pachymetry or conventional ultrasound pachymetry were used for CCT measurements. These methods have poor reproducibility and yield large deviations because of relatively high interobserver and interinstrument variability.

In 1999, Konowal et al11 performed a retrospective study on 9 eyes of 9 patients, who developed overt corneal decompensation 3 to 20 weeks after starting dorzol-
amidine therapy. None of the patients showed improvement in corneal clarity after drug cessation, which demonstrated that the corneal decompensation was irreversible. All of those patients had histories of a compromised corneal endothelial cell layer due to intraocular surgery, such as cataract surgery, trabeculectomy, penetrating keratoplasty, or due to Fuchs endothelial cell dystrophy. The authors suggested that dorzolamide may be contraindicated in patients with “borderline” corneal endothelial function. Correspondence following this article stated that among the more common causes of corneal edema were glaucoma surgery, cataract surgery, increased IOP, intraocular inflammation, and contact lens wear. Therefore, the irreversible corneal decompensation in these cases may have been the same with or without dorzolamide.12

To date, the studies on the effects of dorzolamide on the cornea—excluding the one by Konowal et al—have dealt with study populations with an ECC higher than 2000 cells/mm². Those studies assessed CCT either with optical slitlamp pachymetry or ultrasound pachymetry.

The aim of our study was to investigate the effect of intensive short-term dorzolamide application on patients with clinically diagnosed cornea guttata confirmed with endothelial specular microscopy or an ECC lower than 1400 cells/mm². The laser interferometric biometry technique used in this study detects the small thickness changes of the central cornea induced by dorzolamide better than the relatively coarse optical or ultrasound pachymetry techniques used in other studies.13-17

This research followed the guidelines of the Declaration of Helsinki and was carried out in accordance with the European Union Good Clinical Practice guidelines and according to the Austrian Arzneimittelgesetz. Written informed consent was obtained from all subjects after the nature, scope, and possible consequences of the study had been explained. The ethics committee of the Vienna University School of Medicine (Vienna, Austria) approved the study.

SUBJECTS

Twenty-eight subjects were included in this study: 20 cornea guttata patients, with the typical appearance of the corneal endothelium on slitlamp examination, and 8 healthy age-matched volunteers. The cornea guttata group consisted of 16 women and 4 men, with a mean ± SD age of 76±5 years (range, 69-87 years) and a mean ECC of 1321±339 cells/mm² (range, 875-2250 cells/mm²). The healthy controls consisted of 8 subjects (5 women and 3 men) with a mean ± SD age of 73±6 years (range, 60-80 years) and a mean ECC of 2483±257 cells/mm² (range, 2002-2830 cells/mm²).

Inclusion criteria for the cornea guttata group were age 19 years or older with an ECC of less than 1400 cells/mm², or cornea guttata confirmed by endothelial specular microscopy. Fifteen patients in the cornea guttata group had an ECC of less than 1400 cells/mm² and 5 patients had an ECC of more than 1400 cells/mm² on slitlamp examination, confirmed by endothelial specular microscopy. The control subjects were matched for age and had to have an ECC of more than 2000 cells/mm² and a morphologically healthy corneal endothelium. The visual acuity of all study subjects was 20/200, and the medical history and physical examination results of all subjects were normal.

Exclusion criteria were relevant ophthalmic diseases, history of hypersensitivity to the trial drug or to drugs with a similar chemical structure, symptoms of a clinically relevant illness in the 3 weeks before the first study day, cataract surgery within 3 months before the first study day, glaucoma, IOP higher than 20 mm Hg, and any topical ocular therapy within 2 weeks before the first study day. Prior to study entry, all patients underwent a complete ophthalmic examination, including refraction, visual acuity testing, slitlamp biomicroscopy, applanation tonometry, and fundus examination.

STUDY DRUGS

The study drugs used in this investigation included dorzolamide (Trusopt 2% eye drops; Merck & Co Inc, Whitehouse Station, NJ), a saline placebo (0.9% saline solution; Fresnius Kabi, Graz, Austria), and a carrier placebo (0.075 mg of benzalkonium chloride in 0.9% saline solution with a pH, density, and osmolarity identical to that of Trusopt). The carrier placebo was produced in the pharmacy of the Vienna General Hospital (Vienna). The density, pH, and osmolarity of Trusopt were analyzed to produce a carrier placebo identical in the parameters ascribed to the carrier substance of dorzolamide. This was done in an effort to exclude the possible effects on CCT caused by the low pH or the preservative agent, benzalkonium chloride. The pH of Trusopt and carrier placebo was 5.36 and 5.20, respectively. The density and the osmolarity were 1.017 and 1.005, and 0.287 and 0.299, respectively.

The study medications were randomized, put into identical drop bottles, and labeled by the pharmacy as follows: patient 1, study medication I; patient 1, study medication II; patient 1, study medication III; patient 2, study medication I; and so on. Randomization of study medications was performed by the pharmacy. The pharmacy also kept the randomization list in a sealed envelope until the end of the study and data entry.

ENDOTHELIAL CELL COUNT

Specular microscopy was performed using a KONAN Non-Con Robo SP 8000 (Konan Medical Inc, Tokyo Japan). On the first study day, 3 endothelial photographs of each eye of every subject were taken. Endothelial cells were counted, and the mean ECC/mm² was calculated.

PARTIAL COHERENCE INTERFEROMETRY (PCI) MEASUREMENT

The principle of dual-beam partial coherence interferometry has been described previously.14,15,16 During PCI measurements, a fixation light, focused to infinity, is offered directly to the eye examined. This allows scanning of the CCT along the visual axis. Recent publications have showed that PCI measurements have a high reproducibility. The optical A-scan enables submicrometer precision of 0.3 µm for measurements of the CCT, with a high axial resolution of 12 µm.11 Interobserver and intraobserver variability both showed correlation coefficients of 0.999 for this measurement technique.19,20 All scans in this study were performed by the same person. In contrast, in ultrasound pachymetry, the ultrasonic probe must be placed onto the center of the cornea, exactly perpendicular to the corneal surface, to measure the CCT correctly. In all patients and healthy controls, 20 to 30 longitudinal scans were performed at each time point, and the mean values were used for data analysis. The light source used in this study had a center wavelength of λ=855 nm, with a power of about 220 µW at the cornea or an intensity of approximately 572 µW/cm² (averaged over a 7-mm aperture). Such light intensity is allowed for a duration of about 28 minutes.21 The time needed for a single measurement of the CCT is about 0.5 seconds. Since just 20 to 30 measurements were made, the time of continuous illumination was far below the safety limits.
All scans were stored on compact disc and analyzed after the last patient underwent the last measurement. The scans were evaluated with special software developed at the Institute of Medical Physics, University of Vienna. The optical CCT was divided by the group refractive index of the cornea (1.3851) to obtain the geometric CCT.22

STUDY PROTOCOL

This study was conducted in a randomized, placebo-controlled, double-masked, 3-drug cross over design. After written informed consent was obtained, the patient was given his or her consecutive study number, which determined the randomized sequence of the study medications for this patient. On the first study day, the patients arrived in the morning (9 AM), and specular microscopy was performed as described. A baseline CCT measurement was obtained with PCI. The first drop of study medication I was given. Then, the patient was sent home and was asked to continue taking the study medication I every 6 hours, starting 6 hours after the first dose. The next day, at the same time of day as the baseline measurement on the first day (±1 hour), the 24-hour PCI measurement of the CCT was taken. This first trial day was followed by a 2-week washout period. On trial day 2, the same procedure was performed as on trial day 1, using study medication II. The second trial day was also followed by a 2-week washout period. On trial day 3, the same procedure was performed using study medication III.

STATISTICAL METHODS

The precision of PCI biometry was defined as the SD of multiple recorded consecutive measurements of the CCT during investigation. Partial coherence interferometry scans of the CCT were evaluated after all study subjects underwent their last measurement. After all data had been cross-checked and entered into the database, the pharmacy handed over the envelope with the randomization list, the seal was broken, and the medication sequences of the patients were unmasked.

The variables of interest are presented as mean±SD and range. The SAS statistical software system (SAS Institute Inc, Cary, NC) and STATISTICA for Windows (StatSoft Inc, Tulsa, Okla) were used for calculations. The 24-hour CCT changes in the cornea guttata and the control group were assessed by employing general linear models (SAS procedures GLM and MIXED). These allowed the flexible specification and statistical testing of medication, period, and carry-over effects. Pairwise comparisons between medications between and within groups were adjusted for multiple testing by applying the Tukey-Kramer method. Since the results in the cornea guttata group showed considerable heteroscedasticity, the SAS procedure MIXED was employed to specify an accordingly flexible variance structure to improve, among others, the finally reported pairwise comparison results (confidence intervals [CIs] and P values). All P values are results of 2-sided tests. P values less than .05 were considered statistically significant.

RESULTS

Two superimposed PCI scans of one cornea guttata patient at baseline and after 24 hours of dorzolamide treatment are shown in Figure 1. In this patient, the change in optical CCT is 29 µm. Divided by the group refractive index of the cornea (1.3851), the obtained change in geometric CCT during the time span of 1 day is 21 µm in this patient. Due to the much higher refractive index change at the air/cornea interface compared with the cornea/aqueous humor interface, the signal reflected at the anterior corneal surfaces is more intense—approximately 20 times higher—compared with the one reflected at the posterior corneal surfaces. Hence, the signal from the anterior corneal surfaces is clipped, resulting from saturation of the detector. The pedestal of this signal—the so-called coherence function, which is the Fourier transformation of the light source power spectrum—is more pronounced. In some cases, a slight asymmetry in the pedestal, caused by the spectrum of the light source, is detected. This asymmetry is evident in the peak shape detected from the anterior corneal surfaces signal reflection. Therefore, it may lead to the impression that the 2 signals are not exactly overlaid. In this case, the clipped signal peaks have been overlaid to match the same origin. No additional displacement of the signals from the posterior corneal surfaces was therefore introduced. The CCT changes in both groups at baseline and after 24 hours of study medication are presented in Table 1. The CCT changes in both groups from baseline to 24 hours of study medication are shown in Figure 2. No period and no carry-over effects were detected in either of the 2 groups.

The mean CCT changes within 24 hours in cornea guttata eyes treated with dorzolamide, saline placebo, and carrier placebo were 12.0 µm (95% CI, 7.0-17.1 µm), 0.6 µm (95% CI, −1.0 to 2.2 µm), and 1.3 µm (95% CI, −0.1 to 2.6 µm), respectively. The mean CCT changes in healthy controls were 1.0 µm (95% CI, −1.1 to 3.0 µm), −0.3 µm (95% CI, −1.4 to 0.7 µm), and 1.0 µm (95% CI, −0.8 to 2.9 µm), respectively.

In eyes treated with dorzolamide, there was a statistically significant difference in mean CCT change in the cornea guttata group compared with the control group of 11.1 µm (95% CI, 3.1-19.0 µm; P=.002). No significant difference in mean CCT change was found between cornea guttata and control group eyes with saline.
placebo (1.0 µm [95% CI, −1.8 to 3.7 µm]; P = .92), and carrier placebo (0.2 [95% CI, −3.1 to 3.5 µm]; P > .99) treatment, respectively.

Differences in mean CCT change between dorzolamide HCl and saline placebo, dorzolamide and carrier placebo, and between both placebo treatments in the cornea guttata group were 11.4 µm (95% CI, 3.7-19.2 µm; P < .001), 10.8 µm (95% CI, 3.2-18.4 µm; P = .001), and −0.6 µm (95% CI, −3.7 to 2.4 µm; P = .99), respectively. Differences between mean CCT change of dorzolamide vs saline-placebo, dorzolamide vs carrier-placebo, and between both placebo treatments in the control group were 1.3 µm (95% CI, −2.0 to 4.7 µm; P = .85), −0.05 µm (95% CI, −4.1 to 4.0 µm; P > .99), and −1.4 µm (95% CI, −4.4 to 1.7 µm; P = .77), respectively.

No subject experienced adverse events except for slight stinging, burning, or foreign body sensation and tearing, during several minutes after application of study medications. One CCT PCI measurement of 1 control subject in the saline placebo condition could not be evaluated, owing to a low signal-to-noise ratio (Table 1). Central corneal thickness was measured by PCI with an average precision of 0.6 ± 0.2 µm (range, 0.1-1.3 µm). The average precision of the PCI measurements in the patients and controls were 0.6 ± 0.2 µm (range, 0.1-1.3 µm) and 0.6 ± 0.2 µm (range, 0.2-1.2 µm), respectively. In patients with an abnormal corneal endothelium, there was no greater error using the PCI technique.

**COMMENT**

Short-term application of dorzolamide caused a small but statistically significant thickening of the CCT in patients with cornea guttata. In the healthy control group, short-term application of dorzolamide did not induce significant changes in the CCT.

Dorzolamide is a potent inhibitor of CA II, which is found in the corneal endothelium. Therefore, concerns have been raised that the drug may have an adverse effect on corneal endothelial cell function by inhibiting the bicarbonate pump.4 There is evidence against this concern, demonstrated by short- and long-term effect studies, in which no statistically significant changes in CCT were observed.28 However, all of these studies were performed in patients with clinically normal, healthy corneas with an ECC greater than 1500 cells/mm² and a CCT less than 0.68 mm. Only Konowal et al11 reported on the development of irreversible corneal decompensation following dorzolamide use in patients with compromised endothelial cells. The authors mentioned that dorzolamide may be contraindicated in patients with “borderline” endothelial function because of their capability to attenuate the bicarbonate efflux, which could lead to corneal thickening.

In the previous studies of the effect of dorzolamide on CCT, measurements of CCT were performed using
optical slitlamp or ultrasound pachymetry. However, these measurements have a rather poor reproducibility and large interobserver and intersession variability. Our study used a novel, noncontact, optical biometry method following the laser interferometer principle (PCI), which enabled submicrometer precision for CCT measurements. The use of PCI rather than optical or ultrasonic pachymetry added a level of precision that was not used in previous studies. The high reproducibility of this method enabled exact investigation of subtle changes in the CCT after short-term application of dorzolamide HCl for the first time, to our knowledge.

The other major difference between our study and the previous ones is that we selected patients with reduced ECC. Our study showed that there is a statistically significant increase in CCT in patients with a reduced ECC (1321 ± 339 cells/mm²) treated with dorzolamide for 1 day only. Dorzolamide was applied 4 times daily even though the labeled dose regimen recommends 3 times daily. We chose a slightly higher dose regimen as a superstimulus for this short-term study to maximize the effect of CA inhibition. The placebo medications had a slightly different viscosity compared with Trusopt because no hydroxyethylcellulose (HEC) was added as a viscosity enhancer. The contact time of the placebo medications might, therefore, have been slightly shorter than they would have been with HEC. It is theoretically possible but very unlikely that HEC, which is used to enhance the viscosity of Trusopt, is responsible for the effect on CCT shown in this study. The use of 2 placebo medications (saline placebo and carrier placebo), both of which did not cause any significant change in CCT, shows that the low pH and the preservative agent, benzalkonium chloride, did not influence CCT.

The other studies conducted so far have investigated long-term effects of dorzolamide (>1 day). The most plausible reasons for the discrepancy between their results (no significant thickening of CCT) and ours are that the previous studies dealt with study populations with normal ECCs and that the effect of dorzolamide HCl on CCT was less than the reproducibility of the measurement methods used in those studies. It has been discussed that phosphate might replace bicarbonate as a cotransporter substrate. These endothelial pump mechanisms could create a long-term counter-regulatory mechanism to avoid corneal thickening if the hydrogen carbonate generation via CA is inhibited. This possible counter-regulatory mechanism may explain the results in subjects with normal corneas obtained by Kaminski et al., which revealed a small but nonsignificant increase in CCT after 1 day of dorzolamide application and no change on all other study days after longer-term application.

In summary, our study revealed short-term corneal thickening induced by dorzolamide in patients with a compromised corneal endothelial cell layer. This acute corneal thickening was of a magnitude of 12 µm, which is not of clinical relevance. Our results do not indicate that patients with cornea guttata necessarily have a higher risk of corneal decompensation after prolonged use of dorzolamide. The time span of the study treatment was too short to predict CCT changes for longer treatment periods. These findings call for further investigations on the long-term effects of dorzolamide on CCT in patients with endothelial problems, using the sensitive biometry technique employed in this study.

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