Effect of Riboflavin-UVA–Induced Collagen Cross-linking on Intraocular Pressure Measurement

Timo Romppainen,1 Lucas M. Bachmann,2 Claude Kaufmann,1 Christopfb Kniestedt,1 Michael Mrochen,3 and Michael A. Thiel1,4

PURPOSE. Corneal collagen cross-linking (CCL) with riboflavin and ultraviolet A irradiation has recently been introduced for treatment of corneal ectasia. Yet a CCL-induced increase in corneal rigidity may interfere with intraocular pressure (IOP) measurements. In an investigation of the effect of CCL on the accuracy of IOP measurements, IOP readings before and after CCL were compared.

METHODS. Ten human eye bank corneas were de-epithelialized and mounted on an artificial anterior chamber. The hydrostatically controlled reference pressure in the chamber was adjusted from 10 to 40 mm Hg in 5-mm Hg steps. IOP was measured by Goldmann applanation tonometry (GAT; Haag Streit, Konitz, Switzerland), dynamic contour tonometry (DCT; Pascal tonometer; Ziemer Ophthalmics, Port, Switzerland), and the TonoPenXL (TP; Tono-Pen XL, Medtronic, Jacksonville, FL) before and after CCL, which was performed with a 0.1% riboflavin solution and 30 minutes of UVA irradiation.

RESULTS. Before CCL, GAT, and DCT readings showed an excellent concordance with the manometric reference pressure, whereas TP overestimated the true IOP. After CCL, the reliability of IOP readings decreased with all three tonometers. This decrease resulted in a slight overestimation of mean IOP, but there were also some potentially dangerous underestimations in some individual corneas. The mean (±SD) difference between IOP readings after and before CCL was +1.8 (3.5) mm Hg for DCT, +2.9 (6.1) mm Hg for GAT, and +3.1 (8.5) mm Hg for TP (P < 0.002 for DCT versus GAT or TP).

CONCLUSIONS. In this in vitro model on human corneas, CCL resulted in an overestimation of true IOP by all the tested tonometers. Although the magnitude of this effect was small, care should be taken when measuring IOP with GAT after CCL, as it results in less accurate, much more variable IOP readings.


Corneal collagen cross-linking (CCL) using riboflavin (vitamin B) and UVA irradiation has recently been introduced as a novel therapeutic option for the treatment of corneal ectasia, such as keratoconus.1-2 Riboflavin, administered topically to de-epithelialized corneas, serves as a photosensitizer that is activated by UVA light. The light-induced production of oxygen radicals leads to the development of strong chemical bonds between collagen fibrils, thereby stiffening the cornea. An increase in overall rigidity in human corneas of up to 330% has been reported.3 Phase 2 clinical studies have shown that the increased corneal stiffness induced by CCL can prevent progression of keratoconus.7,4 CCL is still at the stage of early clinical evaluation. An important aspect to investigate is the long-term risk profile. Most patients suitable for this new treatment are young and will require lifelong ophthalmic care because of their keratoconus. Hence, to assess the risk profile of this new treatment, it is important to assess how increased corneal rigidity affects routine ophthalmic examinations such as intraocular pressure (IOP) measurements.

In the clinic, IOP is typically measured with tonometers, such as the Goldmann applanation tonometer (GAT; Haag Streit, Konitz, Switzerland), the TonoPenXL (TP; Medtronic, Jacksonville, FL), and the more recently introduced dynamic contour tonometer (DCT; Pascal; Ziemer Ophthalmics, Port, Switzerland). The working principles of applanation tonometers (such as the GAT) and combined applanation and indentation tonometers (such as the TP) are based on the assumption of a standard corneal rigidity. The working principle of contour-matching tonometry (DCT) presumes a corneal elasticity that allows the cornea to assume the shape of the concave tonometer tip. After CCL, which fundamentally alters the corneal biomechanics, the basic assumptions of these different tonometers may be incorrect. In a theoretical model, alterations in corneal rigidity have been shown to exert an even stronger influence on IOP measurements than corneal curvature or thickness, both of which can affect the accuracy of IOP measurements.

Despite its clinical importance, the effect of CCL on the accuracy of IOP measurements has not been investigated. The purpose of this in vitro study was to assess how IOP measurements obtained by GAT, DCT, and TP are affected by CCL.

METHODS

Ten human corneas from our eye bank that were unsuitable for transplantation because of donor serology or low endothelial cell counts were mounted onto an artificial anterior chamber system (Barron, Katena Products Inc., Denville, NJ). Donor age was 64.8 ± 25.8 years (mean ± SD) and ranged from 17 to 84 years. Mean postmortem time (time from donation to experiment) was 37.3 ± 27.5 days. The corneas where kept in storage medium (Optisol GS; Bausch & Lomb, Irvine, CA) until use. The donor eyes were managed in accordance with the tenets of the Declaration of Helsinki for research involving human tissue.

The anterior chamber system was perfused with physiologic saline (BSS Plus; Alcon, Fort Worth, TX) supplemented with 2.5% dextran (dextran of 100,000 to 200,000 molecular weight; Sigma-Aldrich Corp., St. Louis, MO), to minimize the effect of inhomogeneously hydrated corneas on the tonometer readings. Mounted onto the artificial anterior chamber, the corneas were allowed to deswell until the pachymetry reached steady state, after approximately 30 to 60 minutes.

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The reference pressure in the anterior chamber was adjusted hydrostatically by moving infusion reservoir bottles to different levels above the artificial globe. The reference pressure was controlled by connecting a pressure transducer to the inflow tube in direct proximity to the globe (Fig. 1). The infusion bottle was open to the atmospheric pressure to guarantee stable IOP during the measurements.6

The artificial anterior chambers were affixed to a plate of acrylic glass and mounted vertically on the headrest of a standard slit lamp (model BQ 900; Haag Streit, Konitz, Switzerland). The corneal epithelium was removed with dry cotton swabs immediately after the corneas were mounted onto the artificial anterior chamber to standardize the conditions for IOP measurements before and after CCL and to facilitate the diffusion of riboflavin into the corneal stroma.

IOP Measurements

The GAT and DCT devices are mounted on the slit lamp, whereas the TP is a handheld device. The reference pressure in the artificial globe was increased by increments of 5 mm Hg from 10 to 40 mm Hg. At each pressure level, the system was allowed to calibrate for 5 minutes. The first round of IOP measurements was taken. Measurements were taken in a fixed order of DCT/GAT/TP. Three consecutive measurements with each tonometer at each pressure level were taken. After the first round of IOP measurements, the reference pressure was lowered to a level of 15 mm Hg at which CCL was performed as will be described later. Ten minutes after CCL the reference pressure was lowered to 10 mm Hg, and IOP measurements were resumed.

As the artificial anterior chamber was pressurized with a hydrostatic IOP lacking rhythmic perfusion and pulse amplitude, IOP measurements by DCT were only feasible using the performance test mode (rather than the standard IOP analysis algorithm). The performance test mode allows for accurate IOP measurement without assessing the ocular pulse amplitude. Since the automatic quality indicator (Q1–Q5) is not displayed in the performance test mode, we used a noise indication of 0.1 or 0.2 as an alternative assessment of the measurement quality. The TP was calibrated as described by the manufacturer at the beginning of each experiment. Only TP readings with a coefficient of variance of 5% were accepted for the study.

Collagen Cross-linking and IOP Measurement

Corneal CCL

Riboflavin (riboflavin-5-phosphate; Streuli & Co. AG; Uznach, Switzerland) was diluted in 20% dextran-T-500 (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) to a final riboflavin solution of 0.1%. The solution was administered topically for 5 minutes before irradiation and then every 5 minutes during the UVA irradiation. A radiator with UVA-diodes (365 nm) was provided by the IROC (the Institute for Refractive and Ophthalmic Surgery, Zurich, Switzerland). The corneas were irradiated for 30 minutes at 3 mW/cm² (with a diode–corneal surface distance of 5 cm). This method provides a total UVA light dose of 5.4 J/cm².

Statistical Analysis

Within the IOP range of 10 to 40 mm Hg, we calculated the median (range) difference between the manometric reference pressure and the IOP measurements obtained from each tonometer. Then, we calculated the differences between the pressure measurements before and after CCL treatment for all the three measuring devices (Δpressure = pressure_posttreatment – pressure_pre-treatment). Data are expressed as the mean ± SD. The extent of overestimation of the different measurement techniques was evaluated with the nonparametric Wilcoxon signed ranks test. \( P < 0.05 \) was considered significant.

We calculated the intraclass correlation coefficients (ICCs) by using the variance component procedure for each IOP measurement technique, both before and after CCL. First, we calculated the overall ICC across all pressure levels, entering the IOP measurements as the dependent variable, the cornea as the random variable, and the pressure level as a covariable. Then, we calculated the stratified ICCs for each pressure level independently.

To assess the reliability of the experimental setup, we compared the ICCs for the GAT measurements before CCL with the ICCs obtained in an earlier clinical study of normal human eyes. The ICCs in the experimental setup used in this study ranged between 0.65 and 0.97, depending on the IOP (Table 1), which compares well with the ICC of 0.76 reported earlier in vivo.7

Table 1. ICC for Each Tonometer and at Each IOP Level before and after CCL

<table>
<thead>
<tr>
<th>Tonometer</th>
<th>True IOP (mm Hg)</th>
<th>Before CCL</th>
<th>After CCL</th>
</tr>
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<tbody>
<tr>
<td>GAT</td>
<td>10</td>
<td>0.71</td>
<td>0.93</td>
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<tr>
<td></td>
<td>15</td>
<td>0.65</td>
<td>0.95</td>
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<td>20</td>
<td>0.79</td>
<td>0.85</td>
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<td>25</td>
<td>0.91</td>
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<td>30</td>
<td>0.90</td>
<td>0.95</td>
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<td></td>
<td>35</td>
<td>0.85</td>
<td>0.98</td>
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<tr>
<td></td>
<td>40</td>
<td>0.97</td>
<td>0.97</td>
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<tr>
<td>DCT</td>
<td>10</td>
<td>0.38</td>
<td>0.37</td>
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<td>15</td>
<td>0.05</td>
<td>0.12</td>
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<td>0.60</td>
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<td>25</td>
<td>0.38</td>
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<td>30</td>
<td>0.52</td>
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<td></td>
<td>35</td>
<td>0.66</td>
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<tr>
<td></td>
<td>40</td>
<td>0.38</td>
<td>0.83</td>
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<tr>
<td>TP</td>
<td>10</td>
<td>0.41</td>
<td>0.34</td>
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<tr>
<td></td>
<td>15</td>
<td>0.66</td>
<td>0.68</td>
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<td>0.13</td>
<td>0.72</td>
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<tr>
<td></td>
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<td>0.29</td>
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RESULTS

Mean corneal thickness was 816 ± 40 μm (range: 646–925) before CCL and 804 ± 36 μm (range: 630–971) after CCL. When different pressure levels were used, changes in corneal thickness remained within a mean range of 77 μm before and 52 μm after CCL. Pressure readings before and after CCL are shown in Figure 2. In the physiologic pressure range (10–20 mm Hg) IOP measurements obtained by GAT and DCT before CCL were almost identical with the reference pressure in the perfused anterior chamber (median difference with GAT: 0.0 mm Hg; range: −5.0 to +3.0, and with DCT: −0.1 mm Hg; range: −3.8 to +3.6). In contrast, the reference pressure was overestimated by TP (+3.5 mm Hg; range: −10.0 to +16.0).

After CCL, IOP readings obtained by all three tonometers were higher than before treatment (Table 2). Over the entire pressure range, the mean (SD) difference between IOP readings after and before CCL was 1.8 (3.5) mm Hg for DCT, 2.9 (6.1) mm Hg for GAT, and 3.1 (8.3) mm Hg for TP. There was a significant difference between DCT and GAT in regard to overestimation of the reference pressure (P = 0.002 for DCT versus GAT). TP readings were significantly higher compared with DCT (P = 0.001) but not significantly higher than GAT readings (P = 0.84).

Depending on the reference pressure, the ICC for GAT ranged between 0.65 and 0.97 before and 0.85 and 0.98 after CCL. The ICC for DCT ranged from 0.05 to 0.66 before and 0.12 and 0.83 after CCL. ICCs for TP were between 0.13 and 0.66 before and 0.34 and 0.77 after CCL (Table 1).

DISCUSSION

The main finding of this study is that, after CCL, the manometric reference pressure in the artificial anterior chamber was overestimated by all three tonometers tested. However, despite the reported increase in corneal rigidity after CCL of up to 330%, the magnitude of IOP overestimation is rather small (1.8–3.1 mm Hg) and is considerably smaller than the magnitude of overestimation that was expected from theoretical calculations.5
We have used an in vitro model with normal human eye bank corneas mounted on an artificial anterior chamber to investigate the effect of CCL on IOP measurements. The advantage of such an in vitro model is that it allows us to study exclusively the corneal effect of CCL on IOP readings without potentially confounding in vivo factors such as real alterations in IOP due to possible cross-linking effects on the trabecular meshwork or aqueous humor production. Since physiologic variables such as ocular pulse, eye movements, and tear film instability are neglected, a static model allows a more accurate comparison of the reference pressure with the tonometer readings than would be possible in an in vivo model. Furthermore, an in vivo study would require cannulation of the anterior chamber in patients who chose CCL to avoid invasive ocular surgery. However, our in vitro model may harbor five potential disadvantages. First, mounting the cornea on an artificial anterior chamber may influence corneal biomechanics, thereby affecting IOP readings even without CCL. This possible drawback was addressed by calculating and comparing the ICC for the GAT readings before CCL, with the ICC obtained in vivo in human volunteers with normal corneas. The results were very similar in the in vitro model compared those reported in vivo (0.76). This indicates that the in vitro model closely reflects in vivo conditions with regard to static IOP measurements. The fact that the ICC remained high even after CCL indicates that CCL did not affect the validity of the experimental setup. A second potential problem of the in vitro setup is the fact that IOP measurements had to be taken on de-epithelialized corneas to facilitate riboflavin penetration into the corneal stroma. However, the excellent concordance of DCT and GAT readings with the reference pressure before CCL shows that the lack of a healthy epithelium was not a serious confounding factor. The third potential problem is that the IOP readings in the study, which were taken only 10 minutes after the corneas were exposed to UV-A light may not fully reflect the long-term changes in corneal biomechanics. However, CCL is a physical effect occurring within seconds after the formation of free oxygen radicals, and the increase in corneal stiffness is fully accomplished at the end of the 30-minute irradiation period (oral communication with Eberhard Spoël, University Eye Clinic, Dresden, Germany 2006). Hence, it is unlikely that the 10-minute time lag after the cornea was stiffened would have influenced the study result. However, in vivo results obtained during an extended clinical follow-up are necessary to clarify whether a possible remodeling of the corneal biomechanics affecting IOP measurements will occur in the long term. The fourth potential drawback of our in vitro model is that we used normal eye bank corneas rather than keratoconus corneas, which is the main indication for CCL. However, as will be discussed later, corneas with keratoconus confound IOP readings, even without CCL, and therefore the use of normal corneas to study the effect of CCL on IOP measurement is preferable. The fifth limitation is that the thickness of all specimens studied does not represent the clinical trials published so far, which did not report a significant increase in IOP. However, IOP results obtained during an extended clinical follow-up are necessary to clarify whether a possible remodeling of the corneal biomechanics affecting IOP measurements will occur in the long term. The fourth potential drawback of our in vitro model is that we used normal eye bank corneas rather than keratoconus corneas, which is the main indication for CCL. However, as will be discussed later, corneas with keratoconus confound IOP readings, even without CCL, and therefore the use of normal corneas to study the effect of CCL on IOP measurement is preferable. The fifth limitation is that the thickness of all specimens studied does not represent the normal range of 450 to 600 μm in healthy eyes and corresponds even less to thin keratoconic corneas. Furthermore, the depth of CCL’s effect in edematous corneas is unknown. However, the stiffening effect after CCL when handling the cornea with forceps at the end of our experiments was clearly observable in all corneas, regardless of their thickness. Despite these potential limitations, the pressurized anterior chamber model used in this study is a robust model for investigating the effect of corneal CCL on IOP readings.
underestimation of the reference pressure by GAT occurred only in the three most edematous corneas and may therefore reflect a measurement artifact. Without these three corneas, the mean overestimation by GAT would have increased from 2.9 (6.1) to 6.3 (1.2) mm Hg. Therefore, until it is shown otherwise, clinicians should be aware that IOP measurements obtained by GAT after CCL become less accurate because not just an overestimation but potentially a dangerous underestimation may occur.

IOP measurements in keratoconus are difficult to interpret as GAT and TP underestimate true IOP in corneas with a thin stroma.16–18 The exact amount of IOP underestimation in keratoconic corneas depends on the extent of the disease and the corneal thinning.11,12 Standard nomograms for the calculation of IOP in thin corneas suggest that GAT may underestimate true IOP in keratoconus patients by approximately 2 to 6 mm Hg.8,13–15 Hence, the increase in corneal rigidity induced by CCL may compensate for the effect of a thin corneal stroma in keratoconus.

Of interest, IOP readings obtained by DCT are affected by CCL in a fashion similar to the GAT readings, despite the fact that DCT has been designed to measure IOP independent of structural properties of the cornea.16 However, it should be noted that in this study the IOP measurements by DCT had to be taken in the performance test mode, as our artificial eye model was pressurized with a static pressure without the normal ocular pulse amplitude. In the performance test mode, DCT calculates IOP from a static or only slowly changing pressure curve, rather than from the lower turning point of the well-defined rhythmic oscillating pressure curve created by the cardiac pulse.17 IOP calculation in the performance test mode results in lower measurement accuracy that is reflected in the lower ICCs for the IOP measurement with DCT found in this study, compared with the ICCs reported for DCT in vivo when using the normal clinical tonometer settings (0.69).7 It is therefore possible that CCL would have an even lesser effect on in vivo IOP measurements with the DCT being taken in the standard tonometer mode.

IOP readings with TP before and after CCL were the least accurate among the different tonometers. Our TP readings were very similar to the results found in a clinical study on normal eyes, where TP overestimated the true IOP in the lower pressure range and underestimated IOP in the higher pressure range.18 This error was even more pronounced after CCL than before treatment. In general, the accuracy of IOP readings with TP varied considerably, which is also reflected in the very low ICCs.

In conclusion, CCL resulted in a slight overestimation of the mean IOP, but the magnitude of this effect for GAT and DCT was relatively small. Whereas individual DCT readings remained rather unaffected by CCL, the variability of GAT readings increased in some individual corneas, resulting in a potentially dangerous underestimation of IOP. GAT may therefore not be an ideal tonometer for the follow-up of patients after CCL.

References