ORIGINAL ARTICLE

Pharmacological Treatment of Glaucoma and Biomechanical Properties of the Cornea

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SUMMARY

Purpose: To evaluate and compare the impact of the long-term use of medication to reduce intraocular pressure on the biomechanical properties of the cornea.
Material and methods: A study sample of 305 eyes of 154 patients newly diagnosed with primary open angle glaucoma (POAG, n = 68) or ocular hypertension (OH, n = 6) was included in a prospective cohort study. The control group comprised 80 untreated eyes of 40 patients with ocular hypertension and 80 eyes of 40 patients with no ocular pathology. The following parameters were evaluated: intraocular pressure (IOPg, IOPcc), hysteresis (CH), corneal resistance factor (CRF) and central corneal thickness (CCT). The parameters were evaluated at baseline (untreated) and at follow-up intervals of 3, 6, 9 and 12 months. The same schedule was used for the eyes in the control group. Eyes with POAG or OH were divided into two sub-groups, depending on the type of applied medication: prostaglandin analogues, carboanhydrase inhibitors alone or in combination with beta-blockers.
Results: We did not demonstrate any statistically significant difference in hysteresis in patients with newly diagnosed POAG (as yet untreated) in comparison with the normal eyes in the control group ($p = 0.238$). We demonstrated significantly higher values of CRF ($p = 0.032$) and CCT ($p = 0.013$) in the control group of untreated patients with ocular hypertension. This result confirms a higher number of patients with stiffer and thicker corneas. A statistically significant difference of CH and CRF was demonstrated ($p < 0.0001$) in eyes treated by prostaglandin analogues during the follow up period. In these eyes we also demonstrated a reduction of CCT ($p < 0.001$). We did not record any other statistically significant change in the remaining observed parameters.
Conclusion: The increase in CH and CRF may demonstrate a change of the biomechanical properties of the cornea following the long-term use of prostaglandin analogues. The biomechanical properties of the cornea were not impacted upon by carboanhydrase inhibitors. Further studies are required in order to establish the effect of the long-term use of prostaglandin analogues on the accuracy of IOP measurements.

Key words: glaucoma, intraocular pressure, cornea, hysteresis, central corneal thickness

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INTRODUCTION

The biomechanical properties of the cornea – elasticity and viscosity influence the physiological and pathophysiological functions of the cornea. They determine how the corneal tissue shall react to stress or change of shape.
The possibility of measuring biomechanical properties in vivo remains an important aim for certain diagnostic and therapeutic procedures in ophthalmology. At present the biomechanics of the cornea can be measured in clinical practice using an Ocular Response Analyzer (ORA) instrument. The ORA is a noncontact tonometer, which measures intraocular pressure (IOP) independently of central corneal thickness. It also provides parameters which represent the biomechanics of the cornea: Corneal Hysteresis (CH) and Corneal Resistance Factor (CRF). In refractive surgery it is possible to use this method in order to distinguish potential candidates in whom there is a risk of corneal ectasia following LASIK (1), and also to obtain a more precise diagnosis of certain corneal diseases (keratoconus, Fuchs endothelial dystrophy) (2-4).
In the case of primary open-angle glaucoma, a certain pathological link between viscoelasticity and glaucoma is also being considered. In patients with glaucoma, it is conclusively demonstrated that the cornea (5) and sclera (6, 7) have altered biomechanical properties. If intraocular pressure increases, greater stress is placed on these structures. If we consider that we are measuring a thinner and flexible cornea, this could testify also to a thinner and flexible lamina cribrosa. In the region of the lamina cribrosa, axons of the ganglion cells pass from the environment of the inside of the eye, where there is relatively higher pressure, into the environment of the retrobulbar cerebrospinal space, where the pressure is lower. The viscosity and elasticity of the lamina cribrosa is important for balancing the pressure gradient and providing functional support for the axons of the ganglion

cells, in order to prevent an interruption of the axoplasmic flow (8).
The variability of viscoelasticity may also influence the results of measurement of intraocular pressure.
From the perspective of assessment of the efficacy of treatment and compensation of intraocular pressure in complicated cases of patients with glaucoma, it is also important to obtain the most precise possible values. We have been stimulated to compile this study by the as yet not entirely clarified issue of the possible connection between changes in the biomechanical properties of the cornea during the course of treatment with various types of antiglaucomatous drugs. Pharmacotherapy of glaucoma is represented by six main groups of pharmaceuticals, but only carboanhydrase inhibitors and prostaglandin analogues can potentially influence the structure of the cornea. Prostaglandin analogues activate the group of enzymes known as the matrix metalloproteinase (MMP), which are also present in the corneal stroma and may have an influence on the structure of collagen (9, 10). In general they are responsible for the degradation of the extracellular matrix (ECM). Inhibitors of carbonic anhydrase (ICA) may negatively influence the endothelial pump of the cornea (11) by inhibiting the enzyme carbonic anhydrase II, which is present in the cornea and shares in the optimal hydration of the cornea.

METHOD

The prospective cohort study incorporated a study sample of 305 eyes of 154 patients.
The patients with newly diagnosed primary open-angle glaucoma (POAG) and ocular hypertension (OH) were compared with a control group. The control group comprised 80 eyes of 40 patients with ocular hypertension without therapy and 80 eyes of 40 individuals with no ocular pathology.
The patients with newly diagnosed glaucoma or ocular hypertension were included in the study if the clinical finding required the application of treatment to reduce intraocular pressure and they had not yet received treatment.
The elimination criteria for all groups were previous intraocular surgery, laser or surgical treatment of the cornea, degenerative, dystrophic or inflammatory disease of the cornea. Also eliminated from the study were patients who wore contact lenses, patients with a toric refractive error higher than 2 dioptres, patients who apply eye drops over the long term to reduce intraocular pressure, or who apply artificial tears or other anti-inflammatory drugs.

The study sample is composed of three groups.
Group A (n = 74) comprises patients with newly diagnosed POAG (n = 68) and OH (n = 6), in whom therapy was newly applied. 44 women (59.5%) and 30 men (40.5%) were included in the group. The average age at the time of determination of the diagnosis was 62.2 years in women (±10.36), in men 58.5 years (±11.61). In this group patients were divided into two sub-groups, A1 and A2. Sub-group A1 was treated with prostaglandin analogues (n = 45), A2 with carboanhydrase inhibitors separately (n = 12) or in a fixed combination with beta-blockers (n = 17).
Group B (n = 40) comprises patients with ocular hypertension in whom therapy was not required. The group included 21 women (52.5%) and 19 men (47.5%). The average age was 54.4 years in women (±12.5) and 53.5 years (±12.4) in men. Group C (n = 40) comprises healthy individuals with no ocular pathology. Group C included 26 women (65.8%) and 14 men (34.2%). The average age was 57.96 years in women (±12.15) and 52.14 years (±12.61) in men. The patients were assessed following inclusion in the study within a schedule of follow-up visits at an interval of 3, 6, 9 and 12 months. At the initial examination a personal and ocular anamnesis was taken and a complex ophthalmological examination performed. This concerned automated keratometry and refractometry (Nidek ARK-700A), determination of best corrected visual acuity (BCVA) for distance vision (ETDRS optotype table), determination of subjective manifest refraction (spherical composition and astigmatism), biomicroscopic examination of the anterior segment of the eye on a slit lamp, measurement of intraocular pressure using a noncontact ORA tonometer, measurement of corneal thickness using ultrasonic pachymetry (ORA), examination of the angle of the anterior chamber (Zeiss gonioscopic lens), examination of the fundus by indirect ophthalmoscopy with assessment of the C/D ratio on the disc of the optic nerve and examination of the field of vision (Humphrey – Zeiss perimeter).
The resulting curve of measurement of intraocular pressure, hysteresis and corneal resistance using an ocular response analyzer (ORA) were selected according to the quality score. The average was always taken of the values of the four best quality measurements. Similarly, ultrasonic pachymetry was the result of the average of the values of four measurements. The examination of the visual field was conducted after 6 months on the patients with glaucoma and ocular hypertension, in the control group of healthy individuals at the initial and final examination. The results of the measurement of the biomechanical properties of the cornea and intraocular pressure were evaluated statistically by means of a statistical analysis in the temporal development of the individual measured quantities IOPg, IOPcc, CH, CRF, CCT, by the method of correlation analysis and an ANOVA test.

RESULTS

Intraocular pressure IOPg and IOPcc before the commencement of treatment and after 12 months of observation in group A, newly diagnosed and treated POAG and OH, in control groups B and C without treatment, is summarised in tables 1 and 2.
In sub-group A1, 87 eyes were treated with prostaglandin analogues, in the first year the number of observed eyes dropped to 73 (84%). The average value of intraocular pressure IOPg was 21.88 mmHg (±7.7 mmHg) at baseline before the application of treatment, with a maximum IOP value of 56.5 mmHg and minimum value of 16.7 mmHg. The corrected value of intraocular pressure IOPcc did not differ markedly. The average IOPcc value was 22.0 mmHg (±7.2 mmHg), with a maximum IOP value of 43.5 mmHg and minimum value of 16.3 mmHg. The average value of intraocular pressure IOPg at the end of the observed period was 17.6 mmHg (±4.5 mmHg) and IOPcc 19.0 mmHg (±5.8 mmHg). Intraocular pressure IOPg decreased by 4.2 mmHg and IOPcc by 3.0 mmHg in the observed period. Both values of IOPg and IOPcc show a falling trend.
In sub-group A2, 58 eyes were treated with carboanhydrase inhibitors separately or in a fixed combination with beta blockers, in the first year the number of observed eyes dropped to 48 (83%). The average value of intraocular pressure IOPg was 21.7 mmHg

Table 1 Development of intraocular pressure IOPg in groups A1, A2, B and C

IOPg	A1 – 1D	12M	A2 – 1D	12M	B – 1D	12M	C – 1D	12M
Min.	16.7	10.5	17.7	10.1	21.7	15.7	10.1	10
Max.	56.5	27.9	50.4	31.2	40.8	31.3	26.9	22.4
Median	20.8	17.1	20.9	17.7	25.5	21.6	17.4	15.9
Average	21.8	17.6	21.7	18.5	26.4	21.7	17.4	15.6
SD	7.7	4.5	6.9	5.4	4.7	4	3.8	3
Number	87	73	58	48	80	61	80	72

IOPg = intraocular pressure equivalent according to Goldmann, A1 = group of POAG or OH treated with prostaglandin analogues, A2 = group of POAG or OH treated with ICA or ICA and beta blockers, B = group of ocular hypertension without treatment, C = control group
SD = standard deviation, 1D = 1st day, 12M = 12th month

Table 2 Development of intraocular pressure IOPcc in groups A1, A2, B and C

IOPg	A1 – 1D	12M	A2 – 1D	12M	B – 1D	12M	C – 1D	12M
Min.	16.3	10.5	16.5	9.6	21.3	10.8	10.8	12
Max.	43.5	27.9	49.3	34.3	41.5	22.1	29.8	24.6
Median	20.8	17.1	20.6	18	22.9	19.7	17.8	15.5
Average	22	19	21.7	18.7	24.7	18.6	17.5	15.6
SD	7.2	5.8	7.2	5.3	4.2	3.2	4.3	3.4
Number	87	73	58	48	80	61	80	72

IOPcc = intraocular pressure compensated by properties of cornea, A1 = group of POAG or OH treated with prostaglandin analogues, A2 = group of POAG or OH treated with ICA or ICA and beta blockers, B = group of ocular hypertension without treatment, C = control group
SD = standard deviation, 1D = 1st day, 12M = 12th month

(±6.9 mmHg) at baseline before the application of treatment, with a maximum IOP value of 50.4 mmHg and minimum value of 17.7 mmHg. The average corrected value of intraocular pressure IOPcc was also 21.7 mmHg (±7.2 mmHg), with a maximum IOP value of 49.3 mmHg and minimum value of 16.5 mmHg. The average value of intraocular pressure IOPg at the end of the observed period was 18.5 mmHg (±5.4 mmHg) and IOPcc 18.7 mmHg (±5.3 mmHg). Intraocular pressure IOPg decreased by 3.2 mmHg and IOPcc by 3.0 mmHg in the observed period. Both values of IOPg and IOPcc show a falling trend.

In group B, 80 eyes with ocular hypertension remained untreated, the number of observed eyes dropped in the first year to 61 (76%). The average value of intraocular pressure IOPg was 26.4 mmHg (±4.7 mmHg) at the beginning of observation, with a maximum IOP value of 40.8 mmHg and minimum value of 21.7 mmHg. The average corrected value of intraocular pressure IOPcc was 24.7 mmHg (±4.2 mmHg), with a maximum IOP value of 41.55 mmHg and minimum value of 21.3 mmHg. The average value of intraocular pressure IOPg at the end of the observed period was 21.7 mmHg (±4.0 mmHg) and IOPcc 18.6 mmHg (±3.2 mmHg). Both values of IOPg and IOPcc remained above average over the observed period.

In group C, 80 eyes had no ocular pathology, in the first year the number dropped to 72 (90%). The average value of intraocular pressure IOPg was 17.4 mmHg (±3.8 mmHg) at the beginning of observation, with a maximum IOP value of 21.9 mmHg and minimum value of 10.1 mmHg. The average corrected value of intraocular pressure IOPcc was 17.5 mmHg (±4.3 mmHg), with a maximum IOP value of 22.1 mmHg and minimum value of 10.8 mmHg. The average value of intraocular pressure IOPg at the end of the observed period was 15.6 mmHg (±3.0 mmHg) and IOPcc 15.6 mmHg (±3.4 mmHg). The difference in the values of intraocular pressure IOPg and IOPcc over the observed period was minimal.

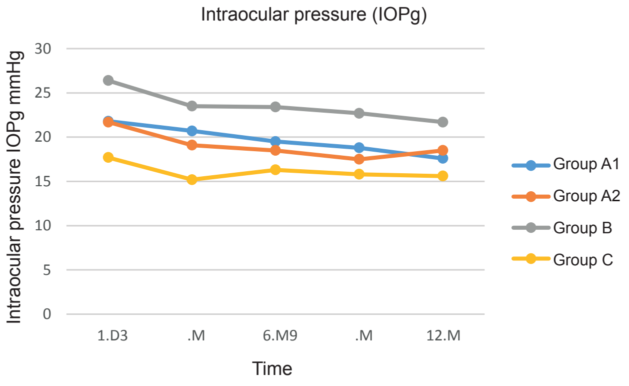
Corneal hysteresis (CH) and corneal resistance factor (CRF) before the commencement of treatment and after 12 months of observation in group A, newly diagnosed and treated POAG and OH, in control groups B and C without treatment, is summarised in tables 3 and 4.

In sub-group A1, the average value of CH was 10.5 mmHg (±2.2 mmHg) at baseline before the application of treatment, and at the end of the observed period 12.8 mmHg (±2.0 mmHg). The increase of the average value of CH was by 2.3 mmHg. The average value of CRF at the beginning of treatment was 11.7 mmHg (±2.2 mmHg) and at the end of the observed period 13.7 mmHg (±1.9 mmHg). The increase in the average value of CRF was by 2.0 mmHg.

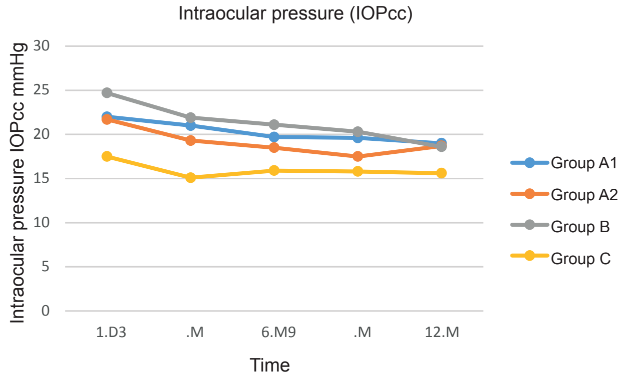
In sub-group A2, the average value of CH was 10.0 mmHg (±2.4 mmHg) at baseline before the application of treatment, and at the end of the observed period 10.2 mmHg (±2.2 mmHg). The average value of CRF at the beginning of treatment was 11.9 mmHg (±2.4 mmHg) and at the end of the observed period 11.1 mmHg (±2.5 mmHg). The difference of the CH and CRF values was minimal.

In group B, the average value of CH was 11 mmHg (±2.7 mmHg) at the beginning of observation, and at the end of the observed period 12.8 mmHg (±3.1 mmHg). The average value of CRF at the beginning of treatment was 14.2 mmHg (±3.0 mmHg) and at the end of the observed period 14.4 mmHg (±3.4 mmHg). The increase in the average value of CH was by 1.8 mmHg, the difference in CRF was minimal.

In group C the average value of CH was 10.5 mmHg (±2.0 mmHg) at the beginning of observation, and at the end of the observed period 10.8 mmHg (±2.0 mmHg). The average



Graph 1 Temporal curve of IOPg in groups A1, A2, B and C.



Graph 2 Temporal curve of IOPcc in groups A1, A2, B and C.

Table 3 Development of CH in groups A1, A2, B and C

CH	A1 – 1D	12M	A2 – 1D	12M	B – 1D	12M	C – 1D	12M
Min.	6.3	7	5.5	4.6	6	8.6	5.2	6.7
Max.	16.2	17.2	17.6	15	18.1	20.3	14.6	14.5
Median	10.3	12.5	9.6	10.4	11	12.2	10.9	10.5
Average	10.5	12.5	10	10.2	11	12.8	10.5	10.8
SD	2.2	2	2.4	2.2	2.7	3.1	2	2
Number	87	73	58	48	80	61	80	72

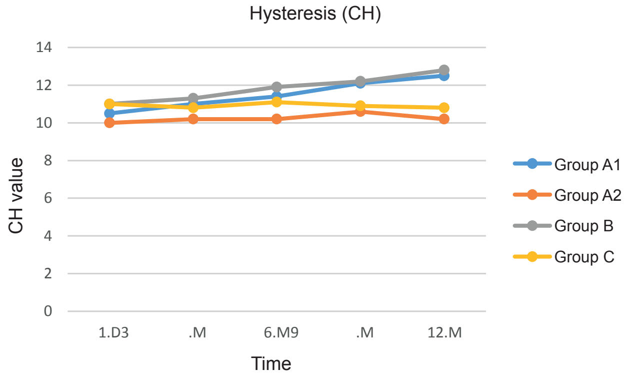
CH = hysteresis, A1 = group of POAG or OH treated with prostaglandin analogues, A2 = group of POAG or OH treated with ICA or ICA and beta blockers, B = group of ocular hypertension without treatment, C = control group
SD = standard deviation, 1D = 1st day, 12M = 12th month

Table 4 Development of CRF in groups A1, A2, B and C

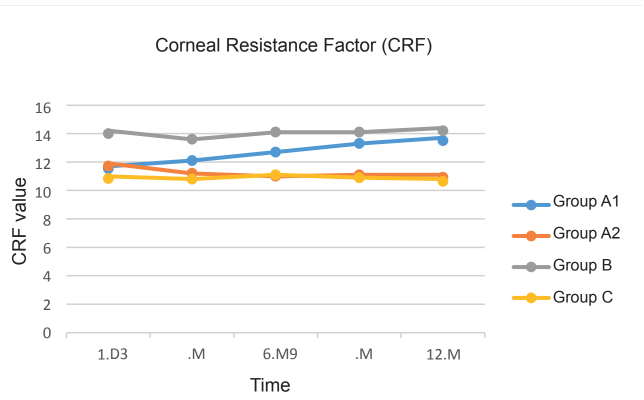
CRF	A1 – 1D	12M	A2 – 1D	12M	B – 1D	12M	C – 1D	12M
Min.	6.8	8.9	7	6.1	9.7	9.9	6.7	5.9
Max.	17.4q	17.9	18.6	17.1	22.1	22.3	14.6	15.2
Median	12	13.9	11.5	10.9	14.2	14.2	11	10.6
Average	11.7	13.7	11.9	11.1	14.2	14.4	11	10.8
SD	2.2	1.9	2.4	2.5	3	3.4	1.9	1.9
Number	87	73	58	48	80	61	80	72

CRF = corneal resistance factor, A1 = group of POAG or OH treated with prostaglandin analogues, A2 = group of POAG or OH treated with ICA or ICA and beta blockers, B = group of ocular hypertension without treatment, C = control group
SD = standard deviation, 1D = 1st day, 12M = 12th month

value of CRF at the beginning of treatment was 11 mmHg (±1.9 mmHg) and at the end of the observed period 10.8 mmHg (±1.9 mmHg). The difference in the values of CH and CRF was minimal.
The resulting temporal curves of CH and CRF are documented by graphs 3 and 4.
Central corneal thickness (CCT) before the commencement of treatment and after 12 months of observation in group A, newly diagnosed and treated POAG and OH, and in control groups B and C without treatment, is summarised in table 5 and graph 5.
In group A1 the average value of CCT dropped over the course of the observation period by -18.8 μm. The increase in the average value of CCT in group A2 was by 14 μm. Control groups B and C manifested minimal differences.
The correlation analysis did not demonstrate a correlation between the quantities IOPcc, IOPg with CH and CRF in any of the groups either at baseline or after one year of observation. The Kruskal-Wallis non-parametric ANOVA (SW STATISTICA) method was used to test the differences between the groups for the quantities IOPg, IOPcc, CH, CRF and CCT at baseline and after 12 months. The test was conducted for the differential quantity after one year as against the baseline value.
Significant differences were demonstrated for the quantity IOPg at baseline between groups A1, A2, B and C ($p < 0.0001$). The differences can be seen from graph 6. The greatest variability of IOPg values is in group A1, whilst in comparison with the other groups, the lowest median is in group C, which confirms the average values of IOP in healthy individuals. Significant differences were demonstrated between groups A1, A2, B and C were also demonstrated for the quantity IOPcc on a 1% level of significance ($p = 0.0001$), analogous to the differences in quantity IOPg. According to graph 7, no significant difference was determined in the



Graph 3 Temporal curve of CH in groups A1, A2, B and C.



Graph 4 Temporal curve of CRF in groups A1, A2, B and C.

Table 5 Development of CCT in groups A1, A2, B and C

CCT	A1 – 1D	12M	A2 – 1D	12M	B – 1D	12M	C – 1D	12M
Min.	470	452	505	509	522	523	470	475
Max.	614	590	625	635	685	685	664	652
Median	556	539	553.5	570.5	607	604	568.5	564
Average	557	538.2	562	576	604.2	601.6	568.8	565
SD	32.4	31.2	31.7	29.7	39.1	40.7	39.1	36.7
Number	87	73	58	48	80	61	80	72

CCT = central corneal thickness, A1 = group of POAG or OH treated with prostaglandin analogues, A2 = group of POAG or OH treated with ICA or ICA and beta blockers, B = group of ocular hypertension without treatment, C = control group
SD = standard deviation, 1D = 1st day, 12M = 12th month

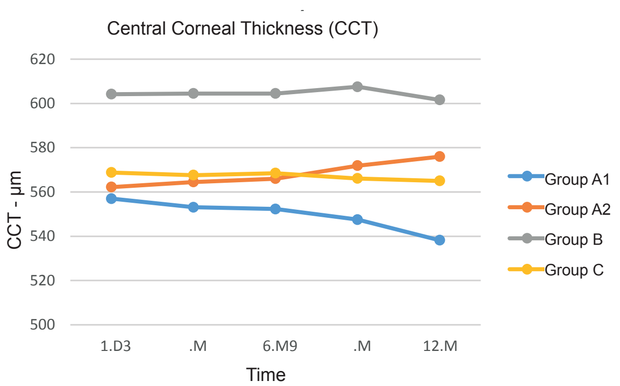
change in intraocular pressure for the quantity IOPg between groups A1, A2, B and C, in comparison with the baseline measurement ($p = 0.6717$). In the majority of patients the inter-year change of IOPg was small, whether it concerned an increase or decrease. A similar conclusion can be formulated also for the quantity IOPcc ($p = 0.503$), as illustrated by graphs 8 and 9.

No significant differences were demonstrated for the quantity CH at baseline between groups A1, A2, B and C ($p = 0.3559$). After one year a significant difference was determined in the change of hysteresis between the groups ($p < 0.001$), group A1 differs markedly – it has the highest median value. This means that in group A1 there was an increase of CH, an increase is also indicated by group B, but in a smaller number of eyes. In the remaining groups A2 and C, the CH value after one year is approximately the same as at the baseline measurement. The difference in the changes is documented by graphs 10 and 11.

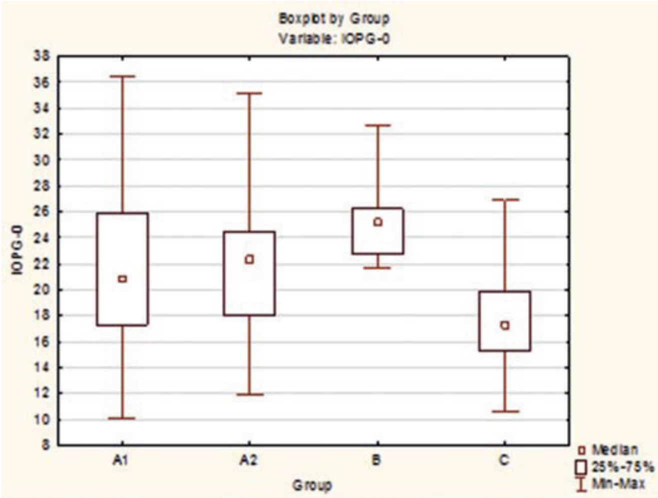
Significant differences were demonstrated for the quantity CRF at baseline between groups A1, A2, B and C ($p = 0.032$). According to graph 12, group B above all manifests different behaviour, acquiring generally higher values than the remaining three groups, which are mutually comparable. Also after one year a significant difference was determined between the groups in comparison with the baseline measurement on a 1% level of significance ($p < 0.001$), as documented by graph 13. Group A1 differs markedly, manifesting the smallest variability of change of CRF, and analogously with the case of CH it has the highest median value. In group A1 there was an increase of CRF, whereas in the remaining groups A2, B and C, the CRF value after one year is approximately the same as at the baseline measurement.

Significant differences were demonstrated for the quantity CCT at baseline between groups A1, A2, B and C ($p = 0.0132$). The greatest variability of CCT values is in group C, group B has a markedly higher median (above the level of 600 μm). After one year a significant difference was determined for the quantity CCT between groups A1, A2, B and C in the change of central corneal thickness in comparison with the baseline measurement on a 1% level of significance ($p < 0.001$). The differences are illustrated by graphs 14 and 15.

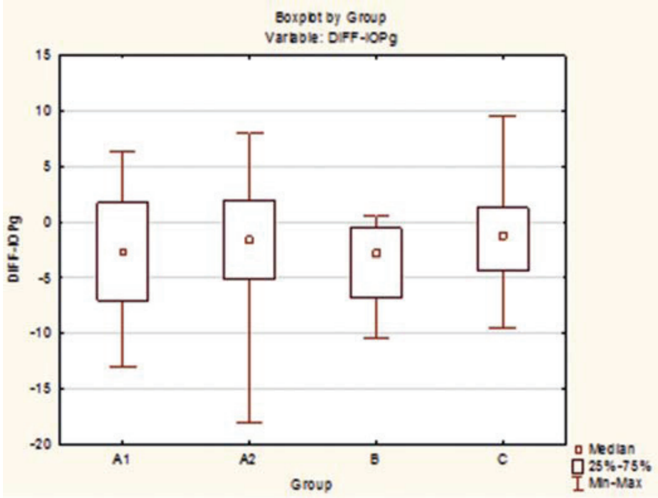
In group A1 the median is negative – in this group there was therefore a reduction in the CCT values after



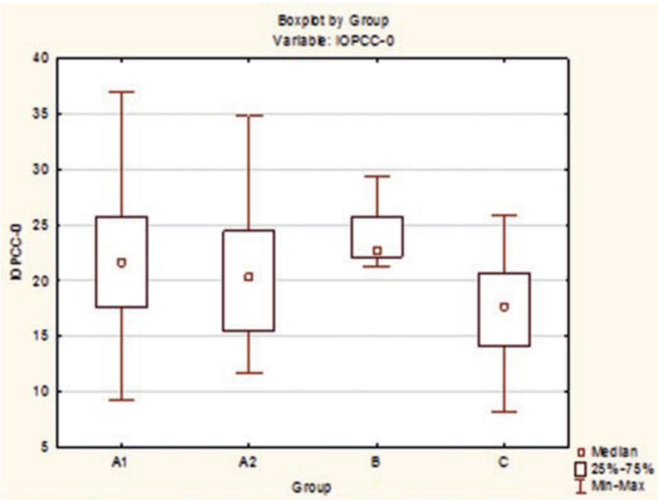
Graph 5 Temporal curve of CCT in groups A1, A2, B and C.



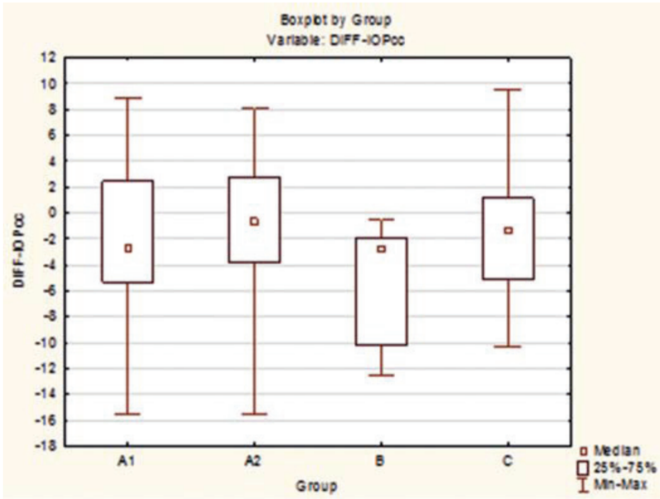
Graph 6 Differences between groups A1, A2, B and C for quantity IOPg at baseline.



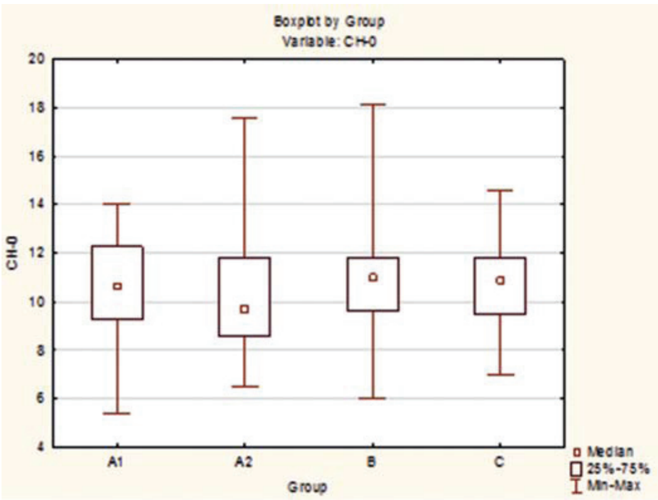
Graph 7 Differences between groups A1, A2, B and C for quantity IOPg after 12 months.



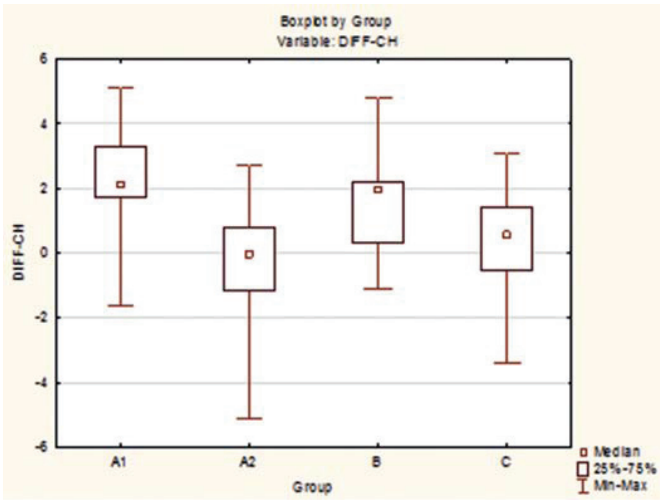
Graph 8 Differences between groups A1, A2, B and C for quantity IOPcc at baseline.



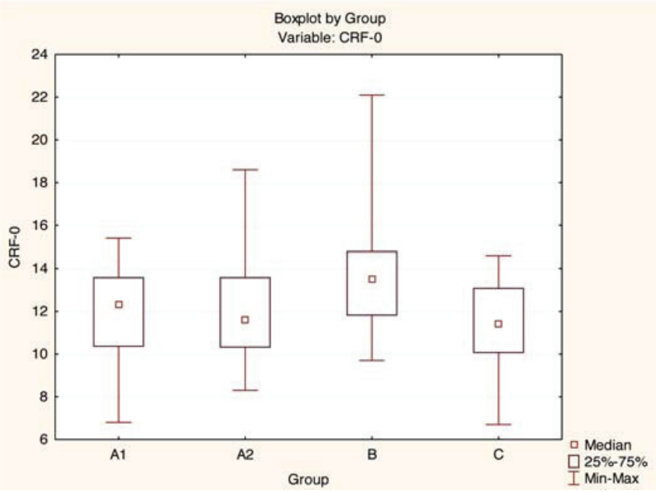
Graph 9 Differences between groups A1, A2, B and C for quantity IOPcc after 12 months.



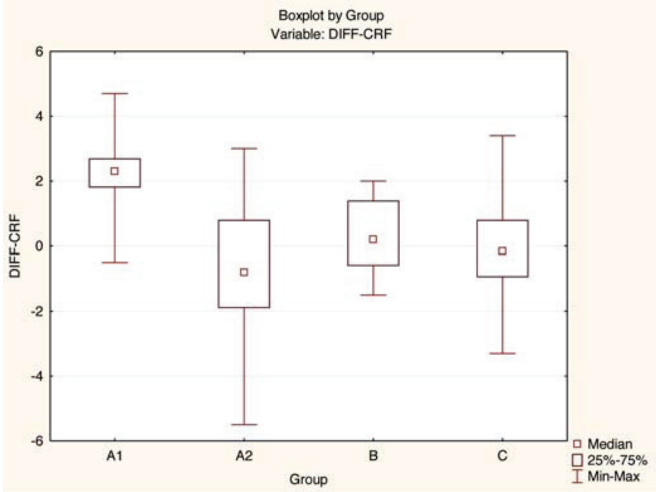
Graph 10 Differences between groups A1, A2, B and C for quantity CH at baseline.



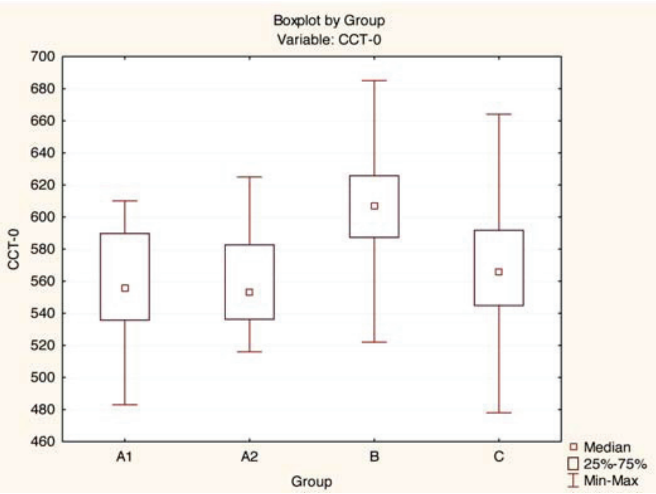
Graph 11 Differences between groups A1, A2, B and C for quantity CH after 12 months.



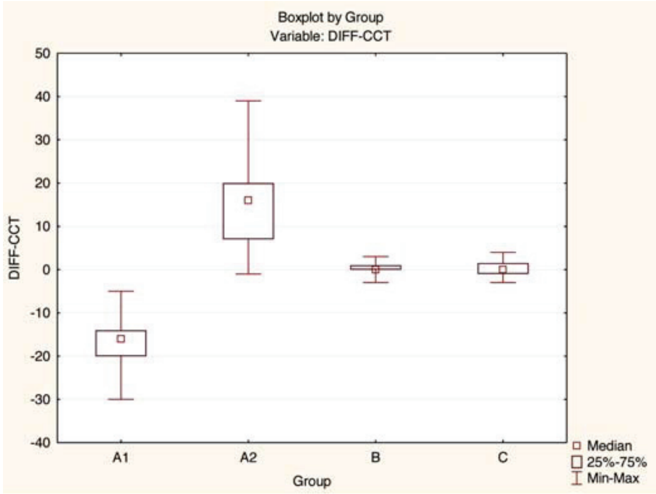
Graph 12 Differences between groups A1, A2, B and C for quantity CRF at baseline.



Graph 13 Differences between groups A1, A2, B and C for quantity CRF after 12 months.



Graph 14 Differences between groups A1, A2, B and C for quantity CCT at baseline.



Graph 15 Differences between groups A1, A2, B and C for quantity CCT after 12 months.

one year (by 5 μm, but also by up to 30 μm). A reduction was recorded in all the patients from this group. The median is 15 μm, which means that in half of the patients the reduction was larger or smaller than 15 μm. By contrast, in group A2 the median is positive – in this group there was therefore an increase in the CCT value after one year. A reduction was recorded in the majority of the patients from this group. On the other hand, this group had the greatest variability of inter-year change in CCT determined in individual patients. Groups B and C are comparable: in both there is very low variability and the median of this differential quantity and the median of this differential quantity is approximately zero in both groups. After one year, CCT in these groups was approximately the same as at baseline.

DISCUSSION

Long-term pharmacotherapy of glaucoma has a favourable influence on the level of intraocular pressure, however, on the other hand it may be accompanied by a whole range of negative effects. The target area of the drugs which lead to a reduction of IOP is the corpus ciliare and the biochemical processes of its enzymes. With regard to the fact that a range of enzymes of the corpus ciliare are present also in the corneal stroma, some of these may also influence biochemical processes in the cornea. This has led to monitoring of the potential negative effects of pharmaceuticals which reduce intraocular pressure on the morphological, metabolic and biomechanical properties of the cornea.

Clinical manifestations of damage to the surface of the conjunctiva and cornea have been known for the greatest length of time in the group of beta blockers. Mietz et al. (12) demonstrated a significant increase in the subepithelial thickness of the collagen band of the conjunctiva in rabbits after 18 months of application of timolol. The negative effect is caused by the active agent of the beta blocker itself, and a toxic effect of the preservative agent was also confirmed.

The most frequently used preservative agent is benzalkonium chloride (BAC). Long-term stress on the surface of the eyes by benzalkonium chloride deteriorates the quality of the lacrimal film, reduces its stability and creates a hydrophobic environment of the surface of the eye. BAC also has toxic effects on the epithelial cells of the cornea and conjunctiva, breaches the haemato-ocular barrier and may be the cause of allergic reaction. A range of studies also compare the effect of beta blockers on the surface of the eyes with and without the preservative agent BAC. The result is more favourable in favour of pharmaceuticals without BAC or with a low concentration thereof (13, 14). Beta blockers and BAC alter the morphology of the corneal epithelium, and do not have an influence on CCT and the biomechanical properties of the cornea.

Carboanhydrase inhibitors are another group of pharmaceuticals which can influence the structure of the cornea. The carbonic anhydrase enzyme in the eyes catalyses the chemical reaction of conversion of carbon dioxide and water into carbonic acid and vice versa. Isoenzyme II, which is important for the dehydration of the stroma, is present in the cornea. The activity of isoenzyme II may be blocked by ICA, which may result in edema and threat to the transparency of the cornea. A number of published studies in the area of this issue confirm the influence of ICAs on central corneal thickness (11, 15-18).

Wirtitsch et al. (16) in their randomised, placebo-controlled, double-blind trial confirmed a statistically significant increase in central corneal thickness after four weeks of application of dorzolamide in patients with Fuchs endothelial dystrophy in the phase of cornea guttata and unchanged central corneal thickness in healthy individuals.

Zhao and co-author (17) referred to two cases of patients in whom decompensation of the cornea occurred after treatment with brinzolamide, in one patient after fifteen months of treatment and in the second after 2 years. A mild edema appeared on the corneas, as well as greying of the stroma, it is of interest that central corneal thickness was unchanged. After discontinuation of treatment the finding disappeared within 3 months.

March et al. (18) published a randomised, double-blind trial in which 372 patients with primary glaucoma were treated with ICAs or beta blockers. After 18 months of treatment they did not demonstrate any changes in central corneal thickness or in the density of the endothelial cells. Similarly, Lester et al. in a multicentric study did not confirm any change of CCT after treatment with ICAs even after treatment with prostaglandin analogues (19).

In our study on patients who were treated with ICAs there was an increase in CCT values after one year. An increase was recorded in the great majority of patients from this group. Although the results of the difference in CCT are statistically significant after twelve months of observation, it is necessary to note the drop in the number of eyes by 17% (48 eyes), in one part of the patients treatment was combined with beta blockers. In addition, the greatest variability of the inter-year change of CCT in individual patients was demonstrated in this group. Miglior (20) states variability of measurement by ultrasonic pachymetry at more than 15 μm, despite the fact that the examination is conducted by the same doctor.

With regard to the fact that there are several factors which can influence CCT (physiological fluctuation of corneal thickness throughout the day, age, level of oestrogen in women), it is not possible to consider the therapy to be unequivocally the sole basis of the changes in corneal thickness. In studies that confirmed an increase in CCT, this mostly concerned corneas with impaired quality of the endothelium (16). In our study sample the endothelium was not evaluated.

Several works focusing on the change of CCT in patients treated with ICAs have been published in the literature (11, 16-18), though little is known about the influence of ICAs on the biomechanical properties of the cornea (21).

In our group treated with ICAs, the values of CH and CRF are approximately the same after one year as at the baseline measurement. The biomechanical properties of the cornea were unchanged.

Prostaglandin analogues and prostamides, currently the most frequently used group of pharmaceuticals, also have the potential to influence the morphological and biomechanical properties of the cornea. Despite the fact that their mechanism of effect on a cellular level has not yet been fully clarified, theoretically their primary effect is explained by an increase of activity of the enzyme matrix metalloproteinase (MMP) type 1, 2 and 3 (22). MMP is a group of 20 types of enzymes which are present in the anterior segment of the eye: in the lacrimal film, in the conjunctiva, in the epithelium, the stroma and endothelium of the cornea, in the scaffold, in the intraocular fluid and the lens. They share in the degradation of ECM particles and basal membranes (23). In patients treated with prostaglandin analogues, the increase in the activity of MMP leads to a reduction in immune response and a subsequent decrease in collagen I, III and IV in the extracellular matrix of the smooth muscle tissue of the corpus ciliare and adjacent sclera. As a consequence of this process, uveoscleral outflow increases, leading to a reduction of intraocular pressure (10). Under the influence of prostaglandin analogues, activation of MMP enzyme was also confirmed in the conjunctiva of rabbits (12), with a subepithelial reduction in the thickness of the collagen band of the conjunctiva (12). A less clarified area is the influence of MMP on the physiological function of keratocytes (fibroblasts) in the corneal stroma.

Wu et al. (9) studied changes in artificially cultivated corneal stromal cells following the application of latanoprost. Immunofluorescent staining demonstrated a change in the biosynthesis of cells, which was manifested in a change in the distribution of collagen type I, a decrease in the migration of cells and a decrease in fibronectin.

Liu et al. (24) detected in an experimental model that artificially cultivated corneal fibroblasts contract following exposure to latanoprost, in contrast with timolol, after which they remain unaffected. There is also a reduction in the gel of the collagen fibres for the production of which fibroblasts are responsible. With regard to the fact that ECM represents a significant part of the volume of the corneal stroma and determines its shape and thickness, a theoretical hypothesis has emerged as to whether prostaglandin analogues alter the volume of ECM and CCT.

Viestenz et al. (25), in a randomised controlled trial, published a reduction of CCT in a group treated with prostaglandin analogues, whilst a control group and a group treated with ICAs did not demonstrate these changes. Certain differences reached up to 60 μm over the course of several months of treatment.

Schlote et al. (26) observed the influence of travoprost on CCT. Within an observation period of 12 months there was a reduction in the first 6 months, in the next 6 months the differences were not significant.

Zhong et al. (27) assessed the influence of prostaglandin analogues on CCT over an observation period of 17.19 ± 15.71 months in 69 eyes of newly treated patients with glaucoma, and demonstrated a reduction of CCT in the case of latanoprost (14.95 ± 5.04 μm), travoprost (15.73 ± 3.25 μm) and bimatoprost (17.00 ± 6.23 μm). The reduction of CCT did not influence changes in the measured values of IOP.

By contrast, a slight increase of CCT following treatment with prostaglandin analogues was determined by Bafa et al. (28). This concerned 108 eyes of patients with newly diagnosed glaucoma treated by prostaglandin analogues (latanoprost, travoprost and bimatoprost). Within the observation period of two years there was a constant increase in CCT by 1.85-8.83 μm in the period of all follow up visits in the group treated with bimatoprost and latanoprost. In the case of travoprost CCT remained unchanged.

In our study on patients who were treated with prostaglandin analogues, there was a reduction in the values of CCT after one year, the median of the values was -15 μm. A reduction was recorded in all patients from this group, in half of these the reduction was greater than 15 μm.

The conclusions ensuing from the studies on the influence of prostaglandin analogues on CCT on patients with glaucoma are equivocal. However, the majority, in accordance with our results, confirm the opinion that prostaglandin analogues may reduce CCT. If there had been a thinning of the cornea due to the influence of prostaglandin analogues and we had evaluated this influence on the precision of measurement of intraocular pressure by applanation separately, the measured results of IOP would have been undervalued.

However, the precision of measurement of IOP is dependent not only on CCT but also on the curvature of the cornea and its biomechanical properties. The resistance which the cornea manifests to deformation during applanation depends more on viscoelasticity and its rigidity than its thickness. From this perspective we focused more on the issue of the influence of prostaglandin analogues on the biomechanical properties of the cornea before and after the application of treatment.

In the group we treated with prostaglandin analogues, a significant difference in the biomechanical properties of the cornea was determined after one year in comparison with the baseline measurement in the quantities of CH and CRF on a 1% level of significance ($p < 0.001$). There was an increase by approximately 2 units. In one half of the patients from this group, the increase was greater than 2 units.

A number of studies have been published in the literature, which explain changes of CH and CRF following the application of treatment by prostaglandin analogues depending on IOP.

In a study on patients with angular glaucoma (29, 30) in whom high IOP values were measured, low CH values were measured and vice versa. Following a reduction of IOP by surgical treatment, there was an increase in the value of CH. Agarwal (31) confirmed this also by observing CH before and after application of treatment by prostaglandin analogues. He came to the conclusion that knowledge of the baseline value of CH before the commencement of treatment with prostaglandin analogues could indicate the size of the reduction in IOP to be expected. In patients where the baseline value of CH is higher, it is possible to expect a smaller reduction of IOP by prostaglandin analogues.

In the study sample we observed this theory was not confirmed, in patients with a higher CH value there was a sufficient reduction of IOP and vice versa. Similarly, a correlation analysis did not demonstrate a mutual dependency of IOPcc and CH ($R = 0.027$) at baseline and ($R = 0.072$) after one year, and between IOPcc and CRF ($R = 0.204$) at baseline and ($R = 0.139$) after one year.

Another potential factor with an influence on the result of CH and CRF may be an error of measurement of CH in the case of extremely high values of IOP. The maximum power of air flow which the ORA instrument is capable of is not enough to ensure sufficient indentation of the cornea and therefore measures a falsely low CH value (32).

It was also determined by means of a more detailed analysis of the measured curves that the instrument is not capable of identifying certain movement of the eye and this dynamic of the bulb may also cause an error of measurement.

And finally, prostaglandin analogues may genuinely influence the structure of the cornea – the structure of the collagen fibres as is demonstrated by experimental studies (10, 24). The viscoelastic properties of the cornea are altered independently of CCT and IOP. A question remains as to whether the ORA instrument has sufficient sensitivity and specificity to record this change reliably.

CONCLUSION

Biomechanical properties of the cornea – according to a range of studies, hysteresis (CH) appears to be a parameter which could predict the progression of glaucoma. It is stated that CH indirectly represents the integrity and viscosity of the lamina cribrosa, and a lower CH value is a risk factor for the progression of glaucoma (33). However, to date the use of the CH value, measured by an ORA instrument, as a risk factor in the progression of glaucoma has its limits. The same CH value may express various proportions of elasticity and viscosity (34).

In the assessed group of eyes with newly diagnosed glaucoma and ocular hypertension, we determined different biomechanical properties of the cornea following the application of treatment in patients treated with prostaglandin analogues.

In the quantities of both CH and CRF, a statistically significant difference was demonstrated after one year in comparison with the baseline measurement ($p < 0.001$). The CH and CRF values increased.

Carboanhydrase inhibitors did not alter the biomechanical properties. The control group of healthy eyes and ocular hypertension without treatment also manifested no change.

In the control group of ocular hypertension without treatment, there was a greater representation of eyes with a rigid cornea and cornea of above-average thickness. This confirmed the importance of measuring CH and CRF in addition to CCT in patients with OH in order to ensure that the therapy is not commenced prematurely.

Although it is not possible to draw unequivocal conclusions from the results of our study, we wished to draw attention to the possible influence of prostaglandin analogues on the biomechanical properties of the cornea during long-term treatment and thus also to the possible influence of the precision of IOP measurement on the course of treatment. At present it is evident that ORA does not differentiate the proportion of elasticity from the proportion of viscosity in the measured CH value. It shall be necessary to conduct further studies in order to confirm the influence on the long-term administration of prostaglandin analogues on the precision of measurement of intraocular pressure using a new generation of instruments, which analyse elasticity and viscosity separately in more detail.

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