UNIVERSITAT POLITÈCNICA DE CATALUNYA
BARCELONATECH
Facultat d'Òptica i Optometria de Terrassa

MÁSTER UNIVERSITARIO EN OPTOMETRÍA Y CIENCIAS DE LA VISIÓN

TRABAJO FINAL DE MÁSTER

ESTUDIO DE LA INERVACIÓN CORNEAL Y LA UNIDAD FUNCIONAL LAGRIMAL EN PACIENTES TRATADOS CON LATANOPROST EN COLIRIO MONODISIS

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La Sra. Gemma Julio Morán como directora del trabajo,

CERTIFICA

Que el Sr. Andrea Da Cortà Fumei ha realizado bajo su supervisión el trabajo "*Estudio de la inervación corneal y la unidad funcional lagrimal en pacientes tratados con latanoprost en colirio monodosis"* que se recoge en esta memoria para optar al título de máster en Optometría y Ciencias de la Visión.

Y para que conste, firmo este certificado.

Sra Gemma Julio Morán

Directora del TFM

Terrassa, 30 de mayo de 2016

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ESTUDIO DE LA INERVACIÓN CORNEAL Y LA UNIDAD FUNCIONAL LAGRIMAL EN PACIENTES TRATADOS CON LATANOPROST EN COLIRIO MONODISIS

RESUMEN

El tratamiento médico crónico es la primera opción para tratar el glaucoma primario de ángulo abierto (GPAA) e hipertensión ocular (OH).

El objetivo de este estudio fue evaluar los efectos del latanoprost sin conservante en las características de la película lagrimal, la sensibilidad corneal y la inervación.

En el grupo de tratamiento se incluyeron 31 ojos de 31 pacientes y 30 ojos de voluntarios sanos sirvieron de controles. Se analizó la sensibilidad corneal, microscopia confocal (IVCM), la osmolaridad lagrimal, el tiempo de ruptura lagrimal, y la prueba de Schirmer I.

Los resultados mostraron una reducción significativa entre el grupo de tratamiento y el grupo control en algunos de los test clínicos. Se encontraron una disminución significativas del número y la densidad de los nervios sub-basal y un aumento significativo de la densidad de las células epiteliales basales en el grupo con tratamiento.

Conclusiones: Los ojos con tratamiento con latanoprost tópico sin conservantes sufrirían pérdida de sensibilidad corneal, disminución del BUT y reducción del número y densidad de los nervios del plexo subbasal de la córnea. La acción proinflamatoria del fármaco u otros excipientes podrían producir estos efectos nocivos sobre la superficie ocular.

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MÀSTER UNIVERSITARI EN OPTOMETRIA I CIÈNCIES DE LA VISIÓ

ESTUDI DE LA INNERVACIÓ CORNEAL I LA UNITAT FUNCIONAL LACRIMAL EN PACIENTS TRACTATS AMB LATANOPROST AMB COLIRI MONODOSI

RESUM

El tractament mèdic crònic és la primera opció per tractar el glaucoma primari d'angle obert (GPAA) i la hipertensió ocular (OH).

L'objectiu d'aquest estudi va ser avaluar els efectes del latanoprost sense conservant en les característiques de la pel·lícula lacrimal, la sensibilitat corneal i la seva innervació.

En el grup de tractament s'inclogueren 31 ulls de 31 pacients i 30 ulls de voluntaris sans van servir de controls. Es va analitzar la sensibilitat corneal, microscopia confocal (IVCM) l'osmolaritat lacrimal, el temps de ruptura lacrimal, la tinció corneal amb fluoresceïna, els símptomes i la prova de Schirmer I.

Els resultats van mostrar una reducció significativa entre el grup de tractament i el grup control en alguns dels tests clínics. Es van trobar un descens significatiu del nombre i densitat dels nervis corneals sub-basals i un augment significatiu de la densitat de les cèl·lules epitelials basals en el grup amb tractament.

Conclusions: Els ulls amb tractament amb latanoprost tòpic sense conservants patirien pèrdua de sensibilitat corneal, descens del BUT i reducció del nombre i densitat dels nervis del plexe subbasal de la còrnia. La acció proinflamatòria del fàrmac o altres excipients podrien produir aquests efectes nocius sobre al superfície ocular.

UNIVERSITAT POLITÈCNICA DE CATALUNYA
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UNIVERSITY MASTER IN OPTOMETRY AND VISION SCIENCE

TEAR FILM CHARACTERISTICS, CORNEAL SENSITIVITY AND INNERVATION IN EYES WITH TOPICAL LATANOPROST WITHOUT PRESERVATIVE

ABSTRACT

Chronic medical therapy is the usual first choice to treat primary open-angle glaucoma (POAG) and ocular hypertension (OH).

The aim of this study was to evaluate the effects of topical latanoprost without preservative on tear film characteristics, corneal sensitivity and innervation.

In the treatment group 31 eyes of 31 patients were finally included and 30 eyes of healthy volunteers served as controls. We analyzed the corneal sensitivity, confocal microscopy (IVCM) tear osmolarity, tear breakup time, corneal fluorescein staining, symptoms and test Schirmer I.

The results showed a significant reduction between the treatment group and the control group in some of the clinical tests. We found a significant decrease in the number and density of corneal sub-basal nerve and a significant increase in the density of basal epithelial cells in the treatment group.

Conclusions: Eyes with chronic treatment with free-preservative topical latanoprost seems to undergo loss of corneal sensitivity, BUT decrease and reduction in the number and length of corneal subbasal nerves. The proinflammatory drug effect or other excipients than preservative could produce this harmful effects on the ocular surface.

COVER LETTER

Dear Editor,

Attached you will find the paper entitled "Tear film characteristics, corneal sensitivity and innervation in eyes with topical latanoprost without preservative", which we are submitting for publication in *Cornea Journal* as an original article.

To evaluate tear film characteristics, corneal sensitivity and innervation in eyes with topical latanoprost without preservative. We analyzed the corneal sensitivity, confocal microscopy (IVCM), tear osmolarity, tear breakup time, corneal fluorescein staining, symptoms and test Schirmer I.

We would be very grateful for any comments or suggestions you may wish to make.

Thank you for your attention.

Yours sincerely,

TITLE PAGE

Title of the article

Tear film characteristics, corneal sensitivity and innervation in eyes with topical latanoprost without preservative

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Keywords

Glaucoma, antiglaucoma drugs, corneal sensitivity, confocal microscopy.

ABSTRACT

PURPOSE: To evaluate tear film characteristics, corneal sensitivity and innervation in eyes with topical latanoprost without preservative.

METHODS: In the treatment group 31 eyes of 31 patients (mean age 63 ± 13; range 35-89) were included, from those, 25 (81%) with POAG and 6 with ocular hypertension. Mean time of treatment was 8 ± 6 months (range= 3-24 months). Thirty healthy eyes served as a control. Corneal sensitivity, tear osmolarity, tear break-up time (BUT), fluorescein staining, symptoms, and Schirmer I test were carried-out. Density of basal epithelial cells, subbasal nerve number and length were measured by in vivo confocal microscopy images.

RESULTS: A significant reduction of the scores was found in the treatment group in corneal sensitivity and BUT and a singificant increase in ocular symptoms of discomfort ($p < 0.05$). No statistically significant differences were found in the rest of studied clinical characteristics.

The density of basal epithelial cells was significantly increased in the treatment group ($p = 0.008$, Student t test). The number of sub-basal nerves was lower in treatment group than in the control group ($p=0.002$, Student t test) and the density of sub-basal nerves was significantly lower in treatment group ($p = 0.005$, Student t test).

CONCLUSIONS: Eyes with chronic treatment with free-preservative topical latanoprost seems to undergo loss of corneal sensitivity, BUT decrease and reduction in the number and length of corneal subbasal nerves. The proinflammatory drug effect or other excipients than preservative could produce this harmful effects on the ocular surface.

MAIN TEXT

INTRODUCTION

Primary open-angle glaucoma (POAG), also known as chronic open-angle glaucoma can be defined as an optic neuropathy characterized by changes in the optic nerve headand visual field deterioration, with or without ocular hypertension.

In general, the primary open-angle glaucoma is an asymptomatic condition. However, abrupt or significant increases in intraocular pressure (IOP) occur with decreased visual field, eye pain and discomfort

Glaucoma is the second leading cause of blindness in the world, the economic and social repercussions are enormous, so today represent a public health problem.[1] Some of the most important factors associated with progression of glaucomatous optic neuropathy are advanced age, a high level of intraocular pressure (IOP), pseudoexfoliation and hemorrhages on the optical disc. About hypertension progression factors, the most importants are: high levels of IOP, advanced age, central corneal thickness and bleeding on the optical disc.[2] Although, IOP plays a very important role in the genesis of this multifactorial disease.

Epidemiological studies suggest that only a tenth part of the patients with elevated pressures have glaucomatous visual field loss. However, about five-sixths of them with glaucomatous disk and changes in the visual field have increased intraocular pressure to 21 mmHg in repeated measurements.[2]

The relationship between IOP and glaucomatous damage is critical to define the treatment of POAG. A vast array of studies support the idea that by reducing IOP is possible to inhibit the rate of progression of glaucomatous neuropathy.

Drug therapy is the first standard option to treat POAG.[2] It reduces the risk of progressive loss of visual field in patients with early or advanced POAG and the development of defects in patients with ocular hypertension (OHT)[3,4]. The most recommended treatment in these cases is a beta-blocker eye drops or prostaglandin analogues such as latanoprost.[2] Glaucoma patients often need to use topical therapy for many years. Hence, the adverse effects of drugs should be minimized to promote compliance with the prescribed treatment and therefore to allow the continuation of the therapy. Several epidemiological studies have shown that eye problems are common in patients treated with topical anti-glaucoma.[4-7] Symptoms of discomfort seem to be associated with a long term use of drug against glaucoma, as it is described, that medication causes changes in the ocular surface and on the morphology of the corneal innervation.[8-10] These adverse effects could be due to the active substance, and preservatives, but the mechanisms involved and the role of each components of topical preparations in inducing toxic or proinflammatory effects is still debated.

The goal of our study is to describe the possible changes in corneal sensitivity, morphology subbasal plexus of the cornea and lacrimal functional unit with the use of unpreserved latanoprost (presentation in single-dose) in patients suffering from POAG or ocular hypertension. The effect of this drugs without the influence of the preservative has not been studied before. This analysis focused in a comprehensive way is essential to establish an objective and individualized therapeutic strategy to reduce the risks of the treatment and to improve the compliance with the results.

METHODS

Patients

A prospective, clinical study was conducted. Before surgery, informed consent was obtained from each patient, and the study was carried out in accordance with the tenets of the Declaration of Helsinki and with the approval of the Ethics Committee of Consorci Sanitari de Terrassa Hospital de Terrassa (Barcelona, Spain).

One eye of each patient was included and the sample was divided into 2 groups according to topical hypotensive therapy. In this way, control group (normal eyes), included 32 eyes of 32 healthy volunteers (13 male, 19 female; mean age,; 60 ± 11; range, 35 to 81 years), who met the following eligibility criteria: absence of current or previous local or systemic disease that could affect the cornea; no history of inflammatory eye disease, including infections; no previous eye surgery; no ocular trauma; no allergic pathology; no topical eye drops, and no contact lens use.

Treatment group included 35 eyes of 35 patients (17 male, 18 female; mean age, 63 ± 14; range, 35 to 89 years), treated with preservative-free topical latanoprost (Monoprost®;Laboratoires Thea, France). The inclusion criteria in this group were as follows: age 18 years or older, diagnosis of POAG or ocular hypertension treated for at least 3 months without changes in the medication used. The following exclusion criteria were used: severe ocular trauma at any time, previous history of intraocular surgery or argon laser trabeculoplasty, current use of contact lenses, history of recent ocular inflammation or infection, previous or current use of other ocular medication including artificial tear therapy, systemic treatment known to affect tear secretion, autoimmune disease, any history or slit-lamp evidence of eye surface disorders.

Clinical investigation

All testing procedures took place at the same time of day, and under temperature and humidity controlled conditions. Detailed biomicroscopic examination of the anterior and posterior segments and ocular adnexa was performed. All measurements were performed under slit-lamp by the same investigator (A.D.C.F). Before doing the tests, all patients make a questionnaire. Questionnaire aimed at assessing, as separate concepts, the participants' self-reported ocular symptoms. Thus, on the one hand, ocular symptoms were explored with a slightly modified version of the Salisbury Eye Evaluation Questionnaire.[11] This six-item questionnaire included questions regarding symptoms of dryness, gritty or sandy sensation, burning sensation, redness, crusting eyelashes and eyes stuck shut in the morning. In addition to these symptoms, we included itchiness, as this symptom is commonly reported by dry eye patients and used in other DEQs.[12] Patients were asked to grade each symptom from 0 to 4 in terms of frequency of occurrence, based on response options: never (0), rarely (1), sometimes (2), often (3) or all the time (4). We chose this questionnaire because it is simple and easy to be self-reported regardless of age or cultural level of the patient, although it does not included a comprehensive list of symptoms.[13] Corneal sensitivity was studied using the Cochet-Bonnet esthesiometer. The monofilament had a diameter of 0.08 mm. The central zone was studied with perpendicular contact and with a length of 60 mm, decreasing in steps of 5 mm if a positive response was not obtained. Two positive responses in three attempts at each filament length were regarded as a positive result. Tear production was determined by the Schirmer I test and Break-up time was used as a measure of tear film stability. Fluorescein sating was carried out to detect injuries in the corneo-conjuntival epithelium. Oxford schema was used to grading them. The Tear break-up time of precorneal tear film after blinking was recorded. Tear break-up time was determined with preservative-free fluorescein drops

in order to avoid irritation.[14] Fluorescence observation contrast was enhanced with a n. 12 yellow Wratten filter and the mean of three consecutive video recorded measurements was used for our analysis. Tear osmolarity was measured using the TearLab Osmolarity System (TearLab Corp, San Diego, CA, USA). The lab-on-a-chip system of the instrument consists of a one-time-use test card, which collects 50 nL of tear sample and analyses it immediately to provide an osmolarity reading.[15] All the eyes were examined with a digital corneal confocal laser-scanning microscope (HRT III Ros- tock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany), a laser scanning in vivo confocal microscopy (IVCM) that uses a 670-nm red wavelength helium neon diode laser source. The confocal laser scanning device uses a X 60 objective water immersion lens and a working distance of 0 to 3 mm from the applanating cap. The images measure 400μm x 400 μm, and the manufacturer specifies an optical section thickness of 4μm. The module uses an entirely digital capture system. In vivo confocal microscopy was performed in the center of the cornea and it was carried out under topical anesthesia with oxybuprocaine 0.4% chlorhydrate (Novesina; Novartis Farma S.p.A., Varese, Italy) instilled in the lower conjunctival fornix before examination. Proper alignment and positioning of the head was maintained with the help of a dedicated target mobile red fixation light for the contralateral eye. A digital camera mounted on a side arm provided a lateral view of the eye and objective lens to monitor the position of the objective lens on the surface of the eye. A drop of 0.2% polyacrylic gel (Viscotirs Gel; Medivis, Catania, Italy) served as coupling medium between the polymethylmethacrylate contact cap of the objective and the cornea. At least 20 images in the central area of the corneal epithelium, sub-basal plexus, and stroma were obtained for each eye. The procedure lasted 2 to 5 minutes. A drop of antibiotic was instilled in the lower conjunctival fornix at the end of each examination. The cornea was then examined by slit-lamp to ensure its integrity.

The confocal images were evaluated in a masked manner, meaning that the investigator did not know to which group the images belonged. The best-focused and most representative images were selected and stored in digital format. The best images of each patient were saved irrespective of the state of the corneal layer and the mean of 3 to 5 images for each parameter was considered for statistical analysis.

The following parameters were evaluated in IVCM images:

1. Epithelial Cell Density: cell density was evaluated in basal epithelium. Basal epithelium was considered 10 µm above Bowman layer. The cell count was performed within a region of interest of standardized dimensions (400µm x 400 µm) using the manual cell counting procedure offered in the software. Cells only partially contained in the area analyzed were not counted. The results were expressed in cells per square millimeter (cell/mm^2)
2. Number of Sub-basal Nerves: this parameter is defined as the sum of the nerve branches present in one image. The image of the sub-basal plexus having the highest number of recognizable nerve fibers was selected for each scan.
3. Density of Sub-basal Nerves: defined as the total length of the nerves visible within a frame (expressed in mm/ mm^2).

STATISTICAL ANALYSIS

After an exploratory analysis, comparisons between groups were carried out applying Fisher's exact test, Mann-Whitney test or Student t test, when appropriate. SPSS V19 was used for statistical analysis and a significant level of $p < 0.05$ was considered. Normal variable distribution was assessed with the Kolmogorov–Smirnov test.

RESULTS

Thirty control eyes of 30 patients completed the study. Mean age of these patients was 60 ± 11; range 35-81years). In the treatment group 31 eyes of 31 patients (mean age 63 ± 13; range 35-89) were finally included, from those, 25 (81%) with POAG and 6 with ocular hypertension. Mean time of treatment was 8 ± 6 months (range= 3-24 months). No significant differences in age and sex were found between groups. Six eyes of 6 patients, initially enrolled (2 eyes in the control group and 4 eyes in the treatment group), were excluded because of incomplete data. The demographic features of control group and treatment group are reported in Table 1.

Table 1. Demographic Features of Control Group and Treatment Group

	Control (n = 30)	Treatment Group (n = 31)	P Value
Age, y, mean ± SD	60 ± 11 (35 to 81)	63 ± 13 (35 to 89)	>0.05
Sex, ♀, ♂	18 - 12	15 - 16	>0.05
Race	caucasian	caucasian	-

Clinical data (Schirmer I test, esthesiometry, tear break-up time, Tear osmolarity, symptoms and fluorescein staining test) for the treatment group and control groups are reported in Table 2, figure 1, 2 and 3. Statistically significant difference was found between treatment and control group in corneal sensitivity (p=0.000, Mann-Whitney test) and Tear break-up (p=0.000, Student t test). No statistically significant difference was found between treatment group and the control group in Schirmer I test and Tear osmolarity (p>0.05; Student t test). A statistically significant difference in the number of symptoms was observed between the control group and the treatment group (p=0.02; Mann-Whitney test). No statistically significant difference was found between treatment group and the control group in fluorescein staining test (p>0.05; Fisher's exact test).

Table 2. Clinical Data Comparison Between Control Group and Preservative-free topical latanoprost Group

	Control	Treatment Group	P Value
Schirmer test, mm, mean ± SD	17 ± 6	15 ± 7	>0.05
BUT, s, mean ± SD	9 ± 5	5 ± 2	<0.05
Tear osmolarity, mOsmol/L, ± SD	310 ± 22	309 ± 20	>0.05

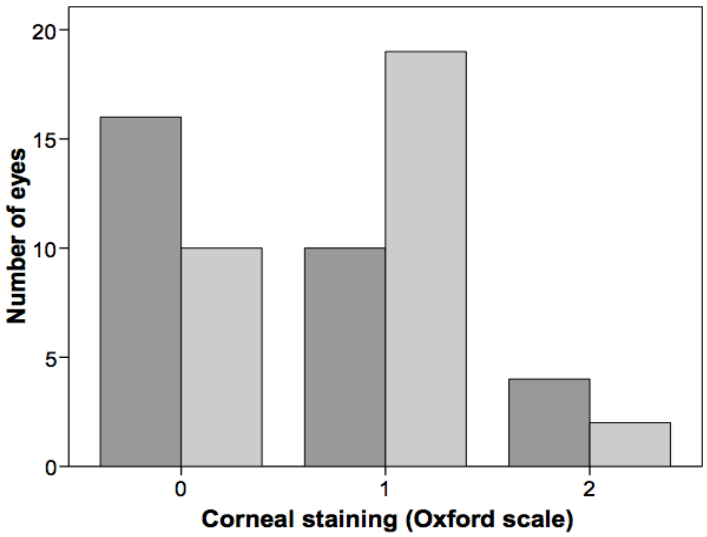

Figure 1. Distribution of the corneal staining reported by the present sample (n = 61)

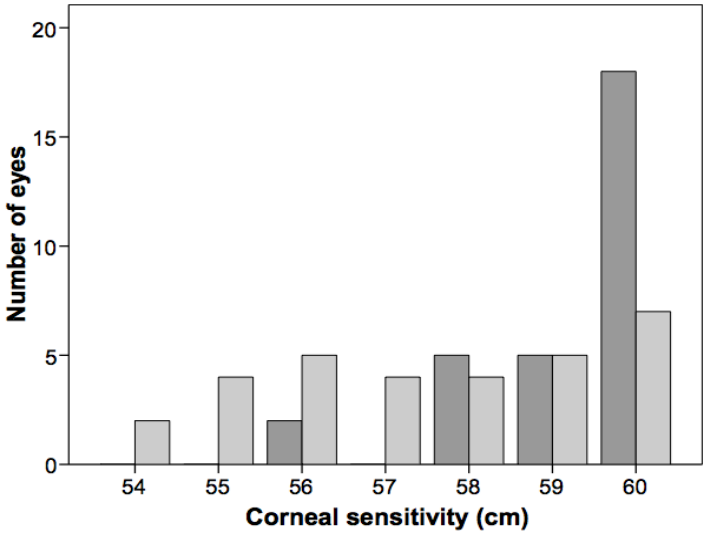

Figure 2. Distribution of corneal sensitivity reported by the present sample (n = 61)

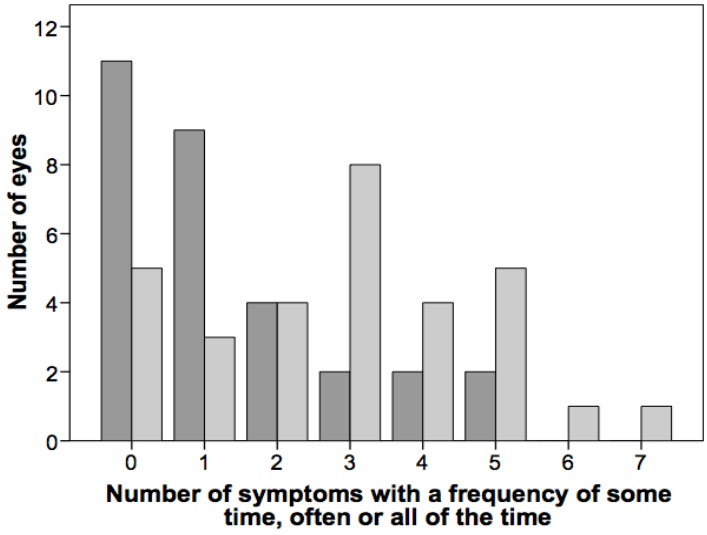

Figure 3. Distribution of the number of symptoms reported by the present sample (n = 61)

In Vivo Confocal Microscopy for the treatment group and control groups are reported in (Table 3). Cell density of the basal epithelium in treatment group revealed a significant increase, with respect to control subjects (P =.008, Student t-test). The basal epithelium density (Figure 4) of treatment group was significantly higher than control group. A significant reduction in the number of sub-basal nerves (Figure 5) was observed between the control group and the treatment group (P=.002, Student t-test). The density of sub-basal nerves (Figure 4) was significantly lower in group of treatment group, with respect to control subjects (P =.005, Student t-test).

Table 3. In Vivo Confocal Microscopy Data Between Control Group and Preservative-free topical latanoprost Group

	Control	Treatment Group	P Value
Basal epithelium, cell/mm^2, (mean ± SD) (minimun to maximum)	4983 ± 588 3880 to 6066	5564 ± 1013 3625 to 7629	<0.05
Number /mm^2, mean ± SD (minimun to maximum)	34 ± 10 12 to 56	26 ± 9 12 to 44	<0.05
Density mm/mm^2, mean ± SD (minimun to maximum)	16 ± 5 4 to 29	13 ± 4 4 to 23	<0.05

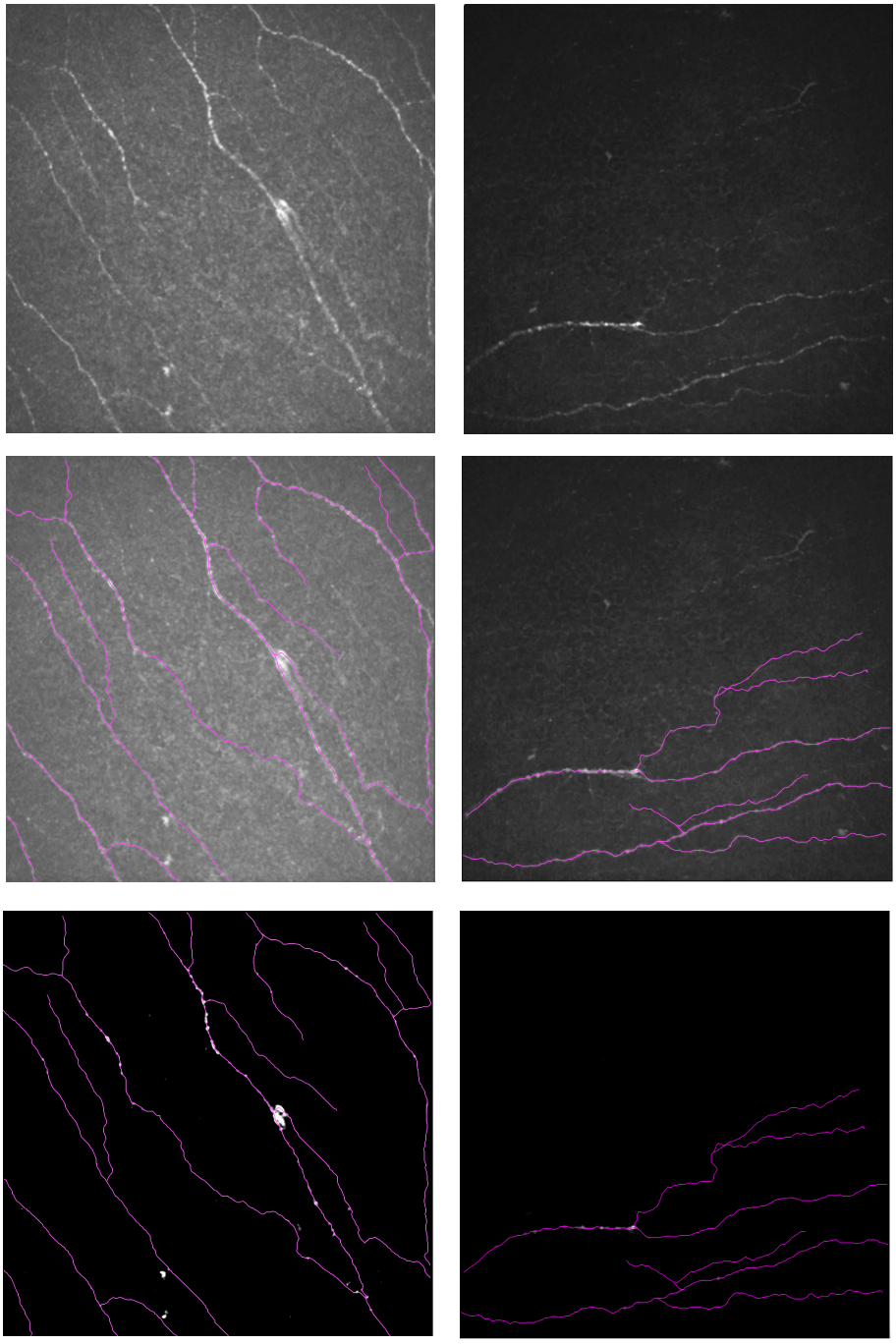

Figure 4. IVCM images (400μm x 400μm) of corneal sub-basal nerves. Examples of sub-basal nerves density and number evaluation. The tracing of subbasal nerves was performed using NeuronJ (pink), a semiautomatic ImageJ plug in to facilitate the tracing and quantification of elongated image structures. Then, the total sub-basal nerves density was measured automatically. Left: control group, Right: treatment group.

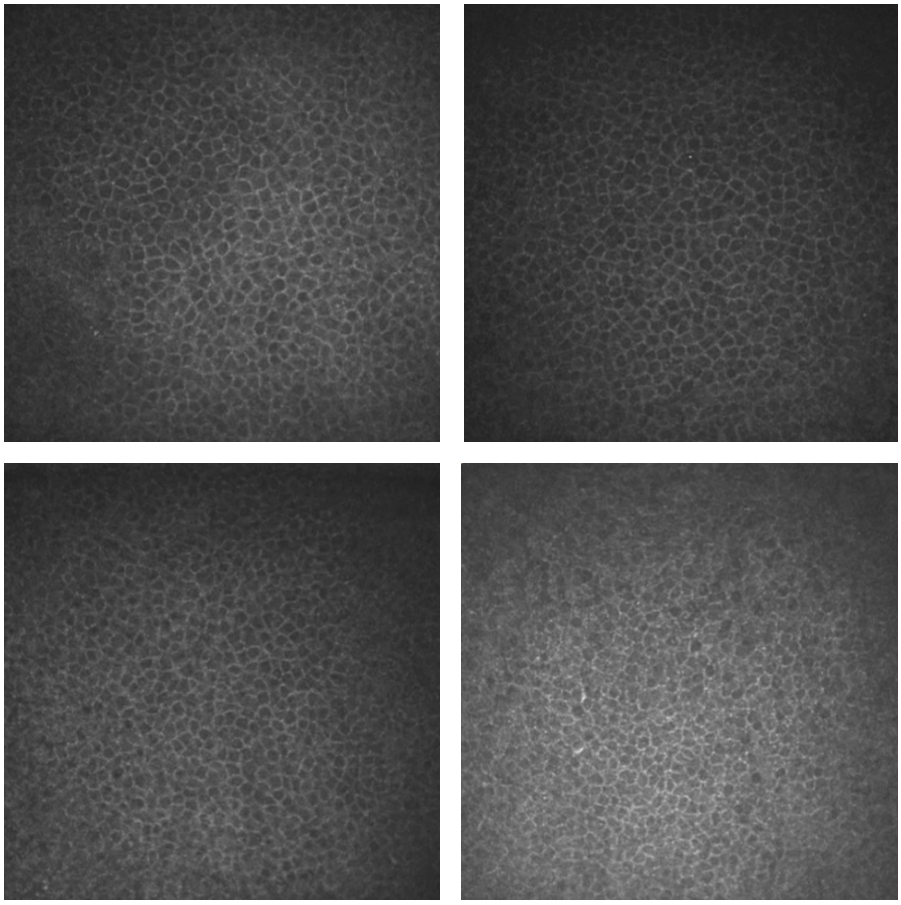

Figure 5. The basal epithelial layer images showed significant increase of cell density in preservative-free topical latanoprost patients with respect to control group. (Top left) control group density = 3928 ±60 cell/mm^2, (Top right) control group density = 4862 ±60 cell/mm^2, (Bottom left) preservative-free topical latanoprost patients group density = 6250 ± 60 cell/mm^2, and (Bottom right) preservative-free topical latanoprost patients group density = 6522 ± 60 cell/mm^2.

DISCUSSION

The aim of the present study was to compare clinical test results and in vivo confocal microscopy features in POAG or ocular hypertension patients on glaucoma therapy with preservative-free topical latanoprost for at least 3 months and in untreated controls. Since current medical treatment for glaucoma requires topical medication for a long period of time, chronic side effects are a major concern. Among these side effects, ocular surface disorders are relatively common, caused either by the drug itself or by preservatives.[16,17] Latanoprost are currently the most commonly prescribed compounds for glaucoma therapy.[17-20] Side effects of latanoprost include cystoid macular edema, choroidal detachment, anterior uveitis, hyperpigmentation of eyelashes and iris, superficial punctate keratitis, and herpes simplex dendritic keratitis.[21-27] These effects could be attributable to the active component as well as to preservatives.[28] The toxic action of preservatives on the eye surface has been widely demonstrated[5] and these side effects may be related to preservative concentration, duration of use, and number of instillations.[7] Ubels and associates demonstrated that preservative-free artificial tear solutions promote recovery of damaged corneal epithelium barrier faster than other commercial artificial tears.[29] Manni and associates showed a significant increase in Interleukin-1β in a group of patients treated with preserved timolol compared to one treated with preservative-free timolol.[30] Noecker and associates showed that antiglaucoma drugs containing low preservative concentrations were associated with less inflammatory infiltrate in the rabbit conjunctiva.[31] Berdy used scanning electron microscopy to compare the effect of 2 preservative-free artificial tear preparations and 0.02% solution of benzalkonium chloride on the corneal epithelium of rabbit eyes: corneas subjected to mild treatment with the benzalkonium chloride solution showed loss of microvilli, increased number of

epithelial holes, and loss of hexagonal shape. Corneas treated with an exaggerated dose of benzalkonium chloride exhibited diffuse cell peeling, retraction of cell membrane borders, destruction of microvilli, and loss of the superficial layer of the corneal epithelium.[32] Many clinical studies on humans confirm these laboratory results. Pisella and associates showed that use of preserved eye drops greatly increases the frequency of ocular irritation in glaucoma patients. The frequency of signs and symptoms was correlated with the number of preserved eye drops used.[5] Baudouin confirmed that the frequency of eye symptoms and signs of ocular surface irritation were higher in patients treated with preserved than preservative-free eye drops and the change from preserved to preservative-free preparation was associated with a significant decrease in ocular irritation.[33] On the contrary, Kuppens and associates demonstrated that tear break-up time was significantly lower in patients treated with preserved and preservative-free timolol than in controls and did not differ significantly from each other, suggesting that the active compound may alter the tear film, while benzalkonium chloride may have other side effects.[34] In our study, the clinical results, excluding the Schirmer I test and Tear osmolarity, showed statistically significant differences between treatment group and control group. Traditional methodological clinical and instrumental diagnostics are unsatisfactory for in vivo study of the ocular surface at cell level, and the use of in vivo confocal microscopy permits a new approach to the study of corneal morphology. In vivo confocal microscopy is not particularly invasive and is quickly performed, safe, and repeatable. In the present study, we performed extensive examination of ocular surface by in vivo confocal microscopy to investigate the toxic effects of chronic glaucoma therapy. Although the morphologic appearances of corneal nerves in ocular surface diseases have been described using these parameters, very few studies have evaluated the relationship between this morphological evaluation and corneal sensation in dry eyes.[35–37] In

addition, only one study has evaluated this relationship in patients treated for glaucoma or ocular hight tension.[38] Our study also offers a systematic evaluation of images, based on parameters that can be analyzed quantitatively. Our evaluation of corneal damage showed that preservative-free latanoprost produced significantly surface damage. The effects of the former were significantly different from that of the control group. Martone and associates demonstrated that the increase density of basal epithelial cells in no-preservative beta-blockers group was not significantly than in the control group.[9] Nevertheless, Roman, Meda and associates in involving animal and human scleral tissues exposed to prostaglandins demonstrated significant matrix metalloproteinases up-regulation and tissue inhibitors of matrix metalloproteinases down regulation with the corresponding altered gene expression, suggesting that prostaglandins stimulate extracellular matrix degradation of ocular surface tissue by modulating the balance between these enzyme. [39,40] Also, in our study the increase density of basal epithelial cells, showed statistically significant differences between treatment group and control group. This epithelial cell modification could determine the stromal changes. In fact, the inflammatory process involving the eye surface could induce stromal apoptotic phenomena and increased stromal proteolytic activity. This in turn could stimulate proliferation leading to keratocyte activation and secretion of neural growth factors contributing to changes in nerve number and shape. Indeed, patients on glaucoma therapy had fewer sub-basal corneal nerves than controls. Nerve fibers are important for corneal trophism and help maintain a healthy corneal surface[41,42] and the lower number and density of nerves in the sub-basal level may explain the lower corneal sensitivity observed in treatment group. It is interesting that the reduced number of nerves of sub-basal fibers was correlated with corneal hypoesthesia and reduced tear secretion. The number of nerves observed in treatment group found in our study is similar to those of Grupcheva and associates[43] and

Oliveira-Soto and Efron.[38] As several studies have demonstrated equivalent efficacy of IOP-lowering medications in the presence and absence of preservatives, the selection of ocular hypotensive drugs containing formulation components with low levels of cytotoxicity may reduce damage to the conjunctiva and cornea, especially over the course of chronic treatment. Exclusive use of preservative-free eye drops or even a reduction in the number of preserved eye drops used reduce the signs of ocular surface irritation in glaucoma patients.[6] There are some issues to consider before drawing any final conclusions. One of the limitations of this study is that it is not a clinical trial and that a double-masked analysis was not performed. However, it is important to consider that patients have been diagnosed before starting the study and in this case a double-masked analysis is not possible. In any case, further research will be important to establish more accurately the magnitude of the differences.

Another limitation was that the measurements of in vivo confocal microscopy were made in the center of the cornea. The results may be different in the corneal periphery but with this technique better images are obtained in the center, and the exact recognition of the depth of the optical section in the stroma was not possible.

The development of alternative, nontoxic preservatives and preservative-free preparations has improved and will continue to improve the overall safety profile of IOP-lowering medications. Cytotoxicity in the ocular surface cells is a well-known detrimental effect induced by benzalkonium chloride containing antiglaucoma agents. There are numerous studies describing the advantages of free-preservative formulation in eye drops, but usually studies do not focus on other excipients besides benzalkonium chloride.[44-46] Conjunctival hyperaemia due to vessel dilatation is one of the most common side effects caused by prostaglandins and prostamids [47,48], but otherwise, these compounds are well tolerated in long-term use. Prostaglandins have

even been claimed to act as non-specific cytoprotectors against benzalkonium chloride toxicity.[49] The use of preservative-free antiglaucoma drops is even more important if glaucoma surgery is planned. Filtration surgery for glaucoma may be unsuccessful in patients with a long history of antiglaucoma treatment, especially multidrug treatment. [50] It is suspected that the toxicity of the preservatives contained in antiglaucoma drops has a role in the failure of surgical treatment.[51] To our knowledge, no previous studies have analize the effects of free-preservative prostaglandins on ocular surface. In our study we provide evidence of ocular surface changes with free-preservative prostaglandins. Currently, popularity of free-preservative formulations in ocular drops has increased in the clinics due to their lower side effects and better patient compliance. Therefore, it is important that all, excipients and drugs, in ocular drop formulations should be evaluated, since some of these agents exert detrimental effects on ocular surface. More research is needed into understand aspects such as the toxicity of the active drug and mechanisms of this toxicity.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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