Original article

The effects of ocular hypotensive drugs on the cornea: An in vivo analysis with confocal microscopy


Servicio de Oftalmología, Hospital Universitario de Fuenlabrada, Madrid, Spain

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Purpose: To evaluate the effects of anti-glaucoma treatments containing benzalconium chloride (BAC) on the human cornea.

Methods: A prospective single masked cohort study was conducted on the 50 eyes of 50 patients. The inclusion criteria were recently diagnosed glaucoma or ocular hypertension with previous treatment, or ophthalmologist-prescribed anti-glaucoma therapy, and oral consent to participate in the study. The patients were not randomized, as the ophthalmologist decided the best therapy according to clinical criteria. The patients were divided in 2 cohorts: those exposed to BAC (23 patients), and those not exposed (27 patients). The mean follow-up period was 22 weeks (range 18–30). The change in cell density before and after therapy was measured in basal layer epithelium, basal layer of limbal epithelium and endothelium. The change in stromal reflectivity and the number of nerve branches in sub-basal nerve plexus was also measured. BAC exposure was blinded to the main researcher.

Results: A greater increase in basal layer epithelium cell density was observed in BAC exposed cohort (p < 0.05). No significant differences were detected in the endothelium, limbal cell density, stromal reflectivity, or sub-basal nerve plexus. Age, sex, IOP, active ingredient or BAC concentration did not affect the direction or magnitude of the ocular surface alterations found.

Conclusion: Chronic anti-glaucoma therapy induces changes in the corneal epithelium. Preservative free drops showed less disruption of the ocular surface by confocal microscopy analysis. Further studies should be conducted to evaluate the clinical impact of these histological findings.

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* Corresponding author.
E-mail address: hector.fernan@hotmail.com (H. Fernández Jiménez-Ortiz).

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Efectos corneales de hipotensores oculares que contienen cloruro de benzoalino: análisis in vivo con microscopía confocal

RESUMEN

Objetivo: Evaluar los efectos sobre la córnea humana de hipotensores oculares con cloruro de benzoalino (BAC).

Métodos: Estudio prospectivo de cohortes, simple ciego realizado sobre 50 ojos de 50 pacientes. Los criterios de inclusión fueron: diagnóstico reciente de glaucoma o hipertensión ocular sin tratamiento previo, terapia antiglaucomatosa prescrita por un oftalmólogo y consentimiento para participar en el estudio. Los pacientes no fueron asignados al azar: el oftalmólogo decidió la mejor terapia de acuerdo a los datos clínicos. Se dividieron en dos grupos: uno expuesto a BAC (23 pacientes) y otro no expuesto (27 pacientes). La media de seguimiento fue de 22 semanas (rango 18–30). Se midió el cambio en la densidad celular antes y después de la terapia en: el epitelio basal, la capa basal del epitelio limbal y el endotelio. También se midió el cambio en la reflectividad estromal y el número de ramas del nervio del plexo subbasal. La exposición a BAC era desconocida para el investigador principal.

Resultados: Un mayor aumento en la densidad de la capa de células basales del epitelio se observó en la cohorte expuesta BAC (p < 0,05). No se detectaron diferencias significativas en la densidad del endotelio, las células del limbo, la reflectividad del estroma ni en el plexo nervioso subbasal. Edad, sexo, PIO, principio activo ni la concentración de BAC afectaron el sentido o la magnitud de las alteraciones encontradas en la superficie ocular.

Conclusión: El tratamiento crónico antiglaucomatoso induce cambios en el epitelio corneal. Gotas sin conservantes mostraron una menor alteración de la superficie ocular por análisis de microscopía confocal. Los estudios futuros deben evaluar el impacto clínico de estos hallazgos histológicos.

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Introduction

Open angle primary glaucoma (OAPG) and ocular hypertension (OHT) are generally treated with topical antiglaucomatous drugs as first choice in order to reduce the risk of progressive visual field loss.1 The most usual drugs are prostaglandin analogs, carbonic anhydrase inhibitors, α-agonists and beta blockers.2 However, all these drugs have side effects at the topical and systemic level. The most common first-line drugs are beta-blockers and prostaglandin analogs. The former (for instance, timolol) is associated to increased adverse systemic effects such as bradycardia, fatigue, depression, and impotence.3,4 Prostaglandin analogs have been demonstrated to be efficient to reduce intraocular pressure (IOP) with very little systemic repercussion and in a posology that facilitates compliance.5 These drugs (tafluprost, latanoprost, bimatoprost and travoprost) are currently available as anti-glaucomatous topical medication6 and are recommended as first line of treatment for reducing IOP in OAPG and HTO.7

Precisely poor compliance is one of the major obstacles to slow down the progression of glaucomatosus damage. In order to improve compliance, adverse effects must be reduced. These could be attributed to the active component as well as to preservatives. However, it is known that the frequency of symptoms and objective ocular surface irritation signs are lower when the therapy does not include preservatives.5,8

Benzalconium chloride (BAC) is a preservative used for maintaining sterile topical solutions and has been demonstrated to have adverse effects on the cornea and conjunctiva. BAC can induce cell toxicity and ocular damage in a dosage-dependent form.9 BAC is an antimicrobial agent that denaturalizes proteins and alters cytoplasmic membranes to the point of causing cytolysis. This property has also been utilized for facilitating the penetration of drugs through the epithelium, as a vehicle for antiglaucoma drugs to penetrate the anterior chamber, denaturalizing the corneal epithelium. BAC has been associated with loss of goblet cells,10 increased deposition of subepithelial collagen and infiltration in the substance of inflammatory cells.11 In addition, BAC is present in the majority of commercial topical antiglaucoma treatments. At present, there are only a few BAC-free active components such as tafluprost (Saflutan®, MSD, NJ, USA), timolol maleate (Timabak®, Thea, Barcelona, Spain) and travoprost (Travatan Z®, Alcon, TX, USA). Tafluprost is a preservative-free F2α-agonist prostaglandin.

Confocal microscopy was first described in 1940 by Goldmann, although it became clinically applicable in the eighties with the development of laser in clinical ophthalmology.12 The noninvasive nature of confocal microscopy provides a method for examining cornea in its physiological condition, avoiding artifacts associated with ex vivo studies. This technique allows a longitudinal examination of the cornea in real time.12 In addition to providing qualitative information, confocal microscopy also enables quantitative analysis of cell populations, of the nerve plexus, etc.13 It provides excellent images of the cell components of the cornea and conjunctiva, enabling a quasi-histological analysis of the ocular surface.12,14 The objective of this study is to evaluate the in vivo effects of drugs
containing BAC on the human cornea in long-term use. Even though there are multiple tests about the toxicity of BAC on ocular surface in vitro, very few studies examine its effects in vivo.

Materials and methods

Prospective and single-blind cohort study of 50 eyes of 50 patients with OAG or OHT. The patients were recruited in the General Ophthalmology practices. Each patient was requested to provide verbal informed consent to participate in the study. The inclusion criteria were as follows:

1. Recent diagnosis of OHT, OAG, closed angle glaucoma (CAG), normotensive glaucoma (NG), glaucoma in pseudoexfoliation syndrome or glaucoma in pigment dispersion syndrome.
2. No previous topical treatment for any ocular disease.

The exclusion criteria were the following:

1. Previous ocular, orbital or palpebral surgery.
2. Previous laser trabeculoplasty or iridotomy.
3. Systemic treatment with known repercussion on lacrimal secretion or self-immune disease.
4. Present or past contact lens use.
5. Evidence of ocular surface disorders in anamnesis or biomicroscopy, such as herpes zoster, and conjunctival scars.

The sample size was calculated using the Epidat version 4.0 statistical application (Sergas, Santiago de Compostela, Spain). The objective was to identify a difference in mean values of at least 15% in the basal cell density on the basis of similar studies. Considering the 2 groups of same size and variance equality, a sample size of 42 individuals was regarded as necessary. The study assumed a loss of 20% and therefore 50 patients were included. The eye selected for the study (left or right) was randomized by means of the SPSS version 18.0 application (SPSS, Chicago, IL, USA).

Slit lamp examination, IOP and confocal microscopy analysis were performed before beginning the therapy. The patients were divided into groups according to BAC exposure. All received monotherapy throughout the period of the study.

Cohort 1: included 25 eyes of 25 patients who received therapies with BAC: latanoprost (Xalatan®, Pfizer, NY, USA), travoprost (Travatan®, Alcon, TX, USA) and 0.03% bimatoprost (0.03% Lumigan®, Allergan, CA, USA). All the patients had bilateral disease. Two patients were excluded after not attending the follow-up visit and therefore 23 patients (23 eyes) completed the study.

Cohort 2: 31 eyes of 31 patients who received therapy without BAC or any other preservative according to the technical data of the drug: tafluprost (Saflutan®, MSD, NJ, USA) and 0.5% timolol maleate (Timabak®, Thea, Barcelona, Spain). All the patients had bilateral disease. Four patients were excluded for not attending the follow-up and accordingly 27 patients (27 eyes) completed the study.

All the measurements were taken by the same researcher (the first author) who was not informed of the prescribed therapy. The patients did know it and for this reason, the study was a single blind study. IOP was measured with Goldmann application tonometer.

Confocal microscopy was performed with microscopic digital scanning laser (HRT II, Rostock Cornea Module, Heidelberg Engineering GmbH, Heidelberg, Germany). This device utilizes red helium-neon diode laser at a wavelength of 670 nm. The images were captured in 0.4 mm × 0.4 mm, each section having a thickness of 4 µm. Topical anesthesia with oxibuprocaaine 4 mg/ml 1 mg/ml and tetracaine (Anestésico doble®, Alcon Cusi SA, Barcelona, Spain) was administered in the inferior conjunctival sac fundus prior to the examination. The device lens was completely submerged in 2% Methocel (OmniVision®, Puchheim, Germany).

At least 20 images were taken of each layer studied with the device on the central cornea. The layers were studied in the following order: corneal epithelium basal layer, sub-basal nerve plexus, stroma and endothelium. Subsequently the subject was requested to gaze naturally in order to expose the temporal limbar conjunctiva (with easier image capture than the nasal side) to capture 20 images of the basal layer of the limbar conjunctival epithelium. The procedure involved 4–5 min for each eye. One drop of tobramycin (Tobrex®, Alcon Cusi SA, Barcelona, Spain) was administered at the end of each examination to avoid infections secondary to the manipulation.

The best focused and most representative images of each histological level were selected for statistical analysis. Cell density was obtained by means of a software included in the device: a small area of each image is selected and the sense or neuronal ramifications are manually counted. Subsequently, the software extrapolates the data obtained in that small area to the entire cornea. The unit of measure was cells per square millimeter (cel/mm²). The data collection protocol was as follows:

1. Cell density in basal epithelium (Fig. 1): the image was focused 10 µm above Bowman's membrane. The minimum examined overall surface was 0.021 mm² and at least 80 cells were counted manually. Cells partially contained within the area were not counted. If the minimum area was not available, the measurement was discarded.
2. Reflectiveness of stroma keratocytes (Fig. 2): the image was focused 10 µm below Bowman’s membrane. To improve the reproducibility of the study, the grading proposed by Marton et al. was adopted (Fig. 1). Thus, each patient was classified on 4 possible grades of stromal reflectiveness. At least 3 images focused on the stroma were studied for each patient.
3. Number of sub-basal nerve ramifications (Fig. 3): in order to improve reproducibility, this parameter was defined as the sum of nerve branches present in an image, similarly to the Marton study. The image of the sub-basal plexus was analyzed with the highest number of identifiable nerve fibers.
4. Cell density in conjunctival limbar basal layer (Fig. 4): the overall minimum surface examined was 0.021 mm² and at least 80 cells were counted manually. The cells partially contained in the analyzed area were not counted. If the minimum area was not available, the measurement was discarded.
5. Cell density in the endothelium (Fig. 5): this was considered only if the characteristic hexagonal cells were observed with good quality. The minimum overall surface examined was 0.021 mm² and at least 50 cells were counted manually. Cells partially contained within the analyzed area were not counted. If the minimum area was not available, the measurement was discarded.

The statistical analysis of the difference between values before and after the treatment was performed, comparing variation in cell density, stromal reflectiveness or number of nerves in the sub-basal plexus throughout the treatment period.

The statistical analysis was performed with the SPSS version 18.0 application (SPSS, Chicago, IL, USA). The normal distribution of variables was determined by means of the Kolmogorov–Smirnov test. The quantitative variables were expressed as mean, standard deviation and confidence interval. The mean values were compared with the T for student test for independent samples. Spearman’s correlation test was used to study the associations between quantitative variables. Values having $p < 0.05$ were considered to be statistically significant.

**Results**

Demographic characteristics and clinical data were similar in both cohorts (Table 1). The age distribution of the sample is consistent with the age distribution of glaucoma in the general population. Gender distribution is also consistent with the higher life expectation in women. Age, gender and follow-up time variables did not exhibit significant differences between the 2 cohorts of the study. Six patients were lost for missing the follow-up, but both losses and patients who completed the study were similar in both cohorts.

A relatively high percentage of OHT was observed. This could be explained by the fact that these were first visits, without structural tests. Presumably, many of these cases will be diagnosed for glaucoma (Table 2). No complications referred by patients or detected in the examination were found. Patients received exclusively topical antiglaucoma treatment during

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**Fig. 1** – Corneal epithelial basal layer. Initial examination (top images) of 2 patients. Cells are irregular, well defined and with homogeneous size. The fundus is brighter than in the conjunctival epithelium. Follow-up examination (bottom images) of the same patients. Cells are slightly smaller than in the initial images.
Fig. 2 – Stromal reflectiveness classified in 4 grades. (A) Grade 1 (top left): normal keratocyte nuclei are seen as shining objects over a dark background. Activated/not activated keratocyte ratio < 1/4. (B) Grade 2 (top right): activated/not activated keratocyte ratio < 1/2, with shining nuclei and more visible cellular processes. (C) Grade 3 (bottom left): activated/not activated keratocyte ratio between 1/2 and 1. Large and hyper-reflective nuclei can be appreciated surrounded by inflammatory cells. (D) Grade 4 (bottom right): the activated/not activated keratocyte ratio is close to 1. Large nuclei on hyper-reflective background and abundant inflammatory cells.

Fig. 3 – Sub-basal nerve plexus. Nerve ramifications can be clearly identified and counted manually.
Fig. 4 – Conjunctival limbus. Epithelial cells and Vogt palisades can be identified. Cells are larger and more irregular than that in the corneal epithelium. Rectangular or triangular cells are frequent and the background is darker than in the cornea due to the opacity of subconjunctival tissue.

Fig. 5 – Endothelium. Hexagonal, regular and larger settings are characteristic of this endothelial cell monolayer. Nuclei can be identified as clear spots in the center of each hexagon.

<table>
<thead>
<tr>
<th>Table 1 – Demographic characteristics.</th>
<th>BAC</th>
<th>BAC-free</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of eyes per intention to treat</td>
<td>25</td>
<td>31</td>
<td>56</td>
</tr>
<tr>
<td>Lost eyes</td>
<td>2 (8%)</td>
<td>4 (12.9%)</td>
<td>6 (10.71%)</td>
</tr>
<tr>
<td>Number of eyes with completed study</td>
<td>23 (92%)</td>
<td>27 (87%)</td>
<td>50 (89.2%)</td>
</tr>
<tr>
<td>Male/female</td>
<td>12/11</td>
<td>8/19</td>
<td>20/30</td>
</tr>
<tr>
<td>Age in years</td>
<td>65.17 ± 9.09 (54–78)</td>
<td>66.26 ± 9.49 (47–83)</td>
<td>65.63 ± 9.23 (47–83)</td>
</tr>
<tr>
<td>Follow-up weeks</td>
<td>21.52 ± 4.2 (18–30)</td>
<td>23.56 ± 4.44 (18–30)</td>
<td>22.65 ± 4.5 (18–30)</td>
</tr>
</tbody>
</table>

BAC, benzalconium chloride.
In the age and follow-up time variables, the mean ± standard deviation is indicated, with the range between parentheses.

<table>
<thead>
<tr>
<th>Table 2 – Diagnostic characteristics.</th>
<th>Ocular hypertension</th>
<th>Open angle primary glaucoma</th>
<th>Normotensive glaucoma</th>
<th>Closed angle glaucoma</th>
<th>Pseudo-exfoliative glaucoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyes</td>
<td>26 (52)</td>
<td>12 (24)</td>
<td>6 (12)</td>
<td>4 (8)</td>
<td>2 (4)</td>
</tr>
</tbody>
</table>

In parentheses, percentage of overall number of eyes or patients.
the period of the study and none required surgery or laser therapy (Table 3).

The initial and final IOP were similar in both cohorts, without significant differences between the group exposed to BAC and the one without BAC (Table 4). All the confocal microscopy data fit in the normal distribution determined by the Kolmogorov–Smirnoff test. Each variable is expressed as the difference between the beginning and end of the study in the same patient. The changes before and after the therapy were compared between the samples exposed and not exposed to BAC by means of the T for student test for independent samples (bilateral significance with 2 tails) (Table 5).

The only observed statistically significant difference was increased cell density of the epithelial basal layer in the cohort exposed to BAC (Fig. 6 and Table 5). Limbus cellularity seemed to increase more in those exposed to BAC than in the other group, even though statistically significant differences were not found (p=0.09) (Table 5). No significant differences were found in stromal reflectiveness or sub-basal plexus. There seemed to be diminished cell population in the endothelium of both groups, more acute in the BAC-free group although the difference was not significant (p=0.09).

The stroma analysis did not evidence patients with high reflectiveness at the beginning or after the therapy. The entire sample had corneal stroma without signs of inflammation, corresponding to grades 1 and 2 of the scale. The images of grades 3 and 4 are from the personal files of the authors and do not belong to any patient of the study (Fig. 2).

The effect of each active principle was studied with the Kruskal–Wallis test, without observing differences between them.

The dosage-dependent effect was researched applying Pearson’s correlation test for quantitative variables. BAC concentration and cell density exhibited a slight inverse correlation (r for Pearson −0.369) (Fig. 7), which even so was statistically significant (p<0.01). This suggests that with higher BAC concentration greater epithelial cellularity is observed.

**Discussion**

The toxic action of preservatives on the surface of the eye has been broadly studied and defined in in vitro and in vivo studies in animals. At present there is growing concern about the toxicity of anti-glaucoma medical treatments due to their chronic nature, which means that patients will be exposed to adverse effects for many years. Significant efforts have been made in recent years to develop preservative-free therapies against glaucoma with the same pharmacokinetic properties as those containing preservative.

Studies published to date confirm that BAC acts as a dosage-dependent proinflammatory and proapoptotic agent. There is a large amount of in vitro studies which have

<table>
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<th>Table 3 – Characteristics of therapy.</th>
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<tr>
<td>Active principle</td>
</tr>
<tr>
<td>BAC concentration (mg/ml) Eyes</td>
</tr>
<tr>
<td></td>
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</tbody>
</table>

BAC, benzalconium chloride.
In parentheses, percentage of overall number of eyes or patients.
Table 4 – Intraocular pressure in mmHg prior to therapy and at follow-up appointment.

<table>
<thead>
<tr>
<th></th>
<th>Baseline IOP</th>
<th>IOP at follow-up appointment</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAC</td>
<td>23</td>
<td>24.61 ± 5.070 (22.42–26.8)</td>
</tr>
<tr>
<td>BAC-free</td>
<td>27</td>
<td>23.37 ± 5.009 (20.78–25.4)</td>
</tr>
<tr>
<td>BAC</td>
<td>23</td>
<td>15.39 ± 2.824 (14.17–16.61)</td>
</tr>
<tr>
<td>BAC-free</td>
<td>27</td>
<td>16.33 ± 4.048 (14.51–18.27)</td>
</tr>
</tbody>
</table>

BAC, benzalconium chloride; IOP, intraocular pressure.

Meaning is expressed as standard deviation and 95% confidence interval between parentheses.

Table 5 – Confocal microscopy results.

<table>
<thead>
<tr>
<th></th>
<th>BAC</th>
<th>BAC-free</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell density in basal epithelium</td>
<td>3673.16 ± 312.94 (3344.75–4001.58)</td>
<td>4037.75 ± 740.7 (2859.12–5216.38)</td>
<td>0.5</td>
</tr>
<tr>
<td>Stromal reflectiveness</td>
<td>1.33 ± 0.56 (0.79–1.88)</td>
<td>1.75 ± 0.5 (0.75–2.55)</td>
<td>0.1</td>
</tr>
<tr>
<td>Number of sub-basal nerve ramifications</td>
<td>8.5 ± 2.73 (5.63–11.37)</td>
<td>6.75 ± 2.21 (3.22–10.28)</td>
<td>0.9</td>
</tr>
<tr>
<td>Endothelial cell density</td>
<td>1766.17 ± 284.7 (1467.38–2064.95)</td>
<td>1669.75 ± 384.72 (1057.56–2281.94)</td>
<td>0.2</td>
</tr>
<tr>
<td>Cell density in limbar conjunctiva</td>
<td>2085.33 ± 571.55 (1485.52–2685.15)</td>
<td>2009.5 ± 537.38 (1154.4–2864.6)</td>
<td>0.5</td>
</tr>
<tr>
<td>Data at follow-up appointment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell density in basal epithelium</td>
<td>3955.83 ± 360.94 (3577.04–4334.61)</td>
<td>3750.256 ± 494.34 (2963.634 ± 536.86)</td>
<td>0.019</td>
</tr>
<tr>
<td>Stromal reflectiveness</td>
<td>1.66 ± 0.51 (1.12–2.21)</td>
<td>1.75 ± 0.5 (0.95–2.55)</td>
<td>0.7</td>
</tr>
<tr>
<td>Number of sub-basal nerve ramifications</td>
<td>8.33 ± 4.17 (3.94–12.71)</td>
<td>5.75 ± 1.5 (3.36–8.14)</td>
<td>0.1</td>
</tr>
<tr>
<td>Endothelial cell density</td>
<td>1774.66 ± 207.5 (1556.9–1992.42)</td>
<td>1537.75 ± 273.15 (1103.1–1972.39)</td>
<td>0.4</td>
</tr>
<tr>
<td>Cell density in limbar conjunctiva</td>
<td>2603.16 ± 855.68 (1705.17–3501.15)</td>
<td>2043 ± 194.26 (1733.87–2352.12)</td>
<td>0.1</td>
</tr>
<tr>
<td>Value at follow-up – baseline value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell density in basal epithelium</td>
<td>[-282.66] ± 207.28 ([-500.2] to [-65.13])</td>
<td>224.8 ± 267.08 ([-99.9] to 548.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stromal reflectiveness</td>
<td>[-0.33] ± 0.81 ([-1.19] to 0.52)</td>
<td>0.17 ± 4.62 ([−4.68] to 5.02)</td>
<td>0.3</td>
</tr>
<tr>
<td>Number of sub-basal nerve ramifications</td>
<td>0.17 ± 4.62 ([−4.68] to 5.02)</td>
<td>1 ± 1.82 (1.91–3.91)</td>
<td>0.09</td>
</tr>
<tr>
<td>Endothelial cell density</td>
<td>[-8.50] ± 302.77 ([-326.24] to 309.24)</td>
<td>132 ± 576.17 ([−784.81] to 1048.81)</td>
<td>0.4</td>
</tr>
<tr>
<td>Cell density in limbar conjunctiva</td>
<td>[-517.83] ± 580.28 ([-1126.80] to 91.13)</td>
<td>−33.5 ± 493.34 (−707.14 to 640.14)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Cell density is expressed in cells per square millimeter (cells/mm²). Stromal keratinocyte reflectiveness is graded from 1 to 4. Mean values are expressed with standard deviation and 95% confidence interval between parentheses. Brackets indicate negative numbers.

* Indicates statistically significant differences.

demonstrated that BAC prevents physiological replacement of epithelial cells even after its withdrawal.51 It promotes the activation of lipoxigenase, and syntheses and secretion of eicosanoids, inflammatory mediators and multiple cytokines such as interleukin (IL)-LA, tumor necrosis factor (TNF), IL-8 and IL-10. All these produce irritation, delayed hypersensitivity and allergic reactions.52,23,24 The time that BAC remains in the eye has been estimated at 48 h after a single instillation, as it can accumulate in cell cytoplasm. For this reason, even one daily dose has negative effects the entire day.24 The action mechanism of BAC toxicity is explained by a detergent effect for cell membranes which produces instability of the lachrymal film, cell loss due to activation of inflammatory cascade and immunological reactions.25

Despite the above findings, Khoh-Reiter suggested that the concentration of BAC in commercially available drops may not be sufficient to cause a significant toxicity in real conditions.22 However, not many studies analyzed how BAC affects the human cornea in vivo. Techniques for in vivo analysis of the cornea are slit lamp examination, cornea impression cytology and confocal microscopy.26 This study chose the latter due to its availability in our hospital and because it was utilized in other studies, thus increasing the reproducibility of results.

The objective of this study is to compare the changes observed in confocal microscopy after antiglaucoma treatments with and without BAC. Other clinical variables such as surface symptoms and tear breakup time were not studied. Only histological changes were studied.

The main finding of this study is increased cell density in corneal epithelial basal layer in the group exposed to BAC. This finding is consistent with studies published to date. Marton et al. found increased cell density in epithelial basal layer despite cellularity reductions of the more superficial corneal epithelium layers.24 This could be explained because the toxic effects on the superficial layers could stimulate cell proliferation in the basal layer. Accordingly, the excess of cell density in the BAC group could be an indirect sign of inflammation and cell loss in the superficial layers.

The dose-dependent effect is relevant to determine the magnitude of the effect of any substance. Even though this study was not designed for that purpose, the authors have found a relationship between BAC concentration and cell density increase in the epithelial basal layer. This finding should be confirmed by additional studies.

Endothelial toxicity has been studied in human cell cultures15 and it has been verified that after topical
instillation, it penetrates the anterior chamber.27 Ayaki and Iwasawa found reduced cell feasibility in cultured cornea exposed to BAC in comparison with unexposed cornea.5 BAC concentration detected in the aqueous humor of rabbits was significantly lower than the concentration in commercially available drops. Accordingly, it is not yet known whether the concentration of BAC reached in the anterior chamber is enough to damage the endothelium. This study did not find statistically significant differences in endothelial cellularity before and after therapy, although the results were close to significance. Additional studies with larger sample sizes should elucidate this point.

It has been proposed that some of the toxic effects observed in studies could be due to their active principles. Timolol is known to cause a significant alteration in tear production and epithelial replacement due to the overexpression of IL-1.28 Some prostaglandins such as latanoprost have also been related to ocular surface disorders such as keratitis punctata.29 This study has found comparable results for bimatoprost, travoprost and latanoprost prostaglandins. In addition, differences between tafluprost and timolol were not found. Even though some studies have demonstrated that different active principles have toxic effects per se on the ocular surface, this study has not found such differences. This could be because this study was not designed with that objective and the subgroups were too small to demonstrate differences between active principles.

The results obtained in this study are different from those obtained by Martone et al.6 The authors have not found differences at the sub-basal plexus level or in the stromal inflammation grade, whereas said authors did refer higher inflammation grade and more nerve ramifications in subjects exposed to BAC. The smaller sample size and the lower follow-up time could be the 2 main reasons for these differences.

The follow-up period was 22 weeks, with a range between 18 and 30 weeks. In accordance with the published in vitro studies, this period is sufficient to detect the toxic consequences of BAC on the cornea.5,21,30 Our results suggest that in vitro studies underestimate the regenerating capacity of the cornea. In addition, it has not been studied whether factors such as age, gender and concomitant therapies could increase BAC toxicity.

At present there are several BAC-free topical ocular hypotensive drugs. However, the vast majority of patients received therapies which include BAC.17 New studies should research the clinical repercussion of the corneal effects at the cell and tissue level.22 In order to perform said research, ophthalmology specific symptom and quality of life surveys such as the Ocular Surface Disease Index (OSDI)31 would be very useful, together with non-specific quality-of-life tests such as SF-36. At present, and on the basis of available evidence, it could be accepted that BAC is toxic for the cornea at the cellular and histological level, but studies based on clinical parameters assessing the risk-benefit profile of BAC as a preservative for chronic therapies such as glaucoma are necessary.

**Conflict of interests**

No conflict of interests has been declared by the authors.

**REFERENCES**


