In Vitro and In Vivo Comparative Toxicological Study of a New Preservative-Free Latanoprost Formulation

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Purpose. To compare in vitro, on the human reconstituted corneal epithelial SkinEthics model, and in vivo, using an acute rabbit toxicological model, the effects of a benzalkonium chloride (BAK)-preserved solution of latanoprost and a preservative-free (PF) latanoprost solution.

Methods. In vitro, the three-dimensional (3D) reconstituted human corneal epithelia (HCE) were treated with PBS, BAK-latanoprost, PF-latanoprost, or 0.02% BAK for 24 hours followed or not followed by a 24 hour post incubation recovery period. Cellular viability was evaluated using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test at 24 hours and the apoptotic cells were counted using TUNEL labeling on frozen sections at 24 hours and 24 hours plus 24 hours. In vivo, rabbits received 50 μL of the same solutions, which were applied at 5 minute intervals a total of 15 times. Ocular surface toxicity was investigated using slit lamp biomicroscopy examination, conjunctival impression cytology (CIC), and corneal in vivo confocal microscopy (IVCM). Standard immunohistology also assessed inflammatory CD45-positive cells.

Results. In vitro, BAK-latanoprost and 0.02% BAK induced significant apoptosis in the apical layers that correlated with the significant decrease of cell viability as assessed by the MTT test. PF-latanoprost slightly decreased cell viability and few apoptotic cells were found in the superficial layers, without reaching statistical significance compared with PBS. In vivo, clinical observation and IVCM images showed the lowest ocular surface toxicity with PBS and PF-latanoprost, while BAK-latanoprost and BAK induced abnormal corneoconjunctival aspects. PF-latanoprost showed the lowest CIC score, close to the PBS score and induced fewer CD45-positive cells in both the limbus and the conjunctiva compared with BAK and latanoprost, as assessed by immunohistology.

Conclusions. We confirm that rabbit corneoconjunctival surfaces presented better tolerance when treated with PF-latanoprost compared with the standard BAK-latanoprost preparation or the BAK solution. (*Invest Ophthalmol Vis Sci.* 2012;53:8172–8180) DOI:10.1167/iovs.12-10766

Prostaglandin (PG) analogs have become first line treatments among glaucoma medications. Currently, five different PG analogs, isopropyl unoprostone, latanoprost, travoprost, bimatoprost, and tafluprost, are used for the treatment of glaucoma. These PGs are superior to beta-adrenoceptor antagonists in terms of lowering IOP,[1,2] and they induce no severe side effects during long term clinical use.[3] Among these PGs, latanoprost presents a highly advantageous balance in terms of IOP lowering efficacy and tolerance[2] and is still the mostly frequently used PG analog worldwide. However, a commercial solution of latanoprost is proposed in a preserved formulation, which raises a number of issues, especially in patients with an abnormal or sensitive ocular surface. Benzalkonium chloride (BAK) is the most commonly used preservative in eye drops. Indeed, it has already been shown to exhibit toxic and inflammatory effects in clinical,[4] in vivo,[5] and in vitro studies.[6] In cell culture, BAK induced oxidative effects, including mitochondrial activity and glutathione injury as well as caspase-dependent and -independent apoptosis counteracted by an autophagic process.[3] Recently, using in vitro[7] and in vivo approaches,[8] it has been shown that BAK induced corneal neurotoxicity. Moreover, chronic use of a preservative is responsible for apoptosis of conjunctival cells and conjunctival inflammation that have demonstrated negative effects (e.g., on glaucoma surgery efficacy).[9,10]

New preservative-free eye drops have therefore been developed. They have consistently demonstrated their good tolerance in vitro,[11] ex vivo, and in patients, who disclosed significantly fewer ocular symptoms and signs of irritation, such as pain, discomfort, or dry eye sensation.[12] Single-dose units, such as carteolol,[13] timolol,[14] or tafluprost, as well as Travatan Z, in which BAK has been replaced with the Sofzia self-preserved ionic buffer, are examples of new PG formulations without BAK or without preservative.

We have developed new in vitro and in vivo tools for the assessment of tolerability and toxicity in a three-dimensional (3D) reconstituted corneal epithelial model,[15] and in an experimental model of acute toxicity in rabbits.[16] Our 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test procedure, combined with immunohistologic analyses of human 3D tissue showed increased sensitivity levels and detected slight damage even in the most superficial layers. In vivo confocal microscopy (IVCM) and conjunctival impression cytology (CIC) are significant in vivo and ex vivo tools that are

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reliable for the observation, at a cellular level, of toxic and/or immunoallergic reactions on the ocular surface. They can analyze inflammation and apoptosis in the conjunctival epithelium without the need for sacrificing the test animal. In this study, we combined these two in vitro and in vivo models to evaluate the toxicological profile of PF-latanoprost, a new preservative-free (PF) latanoprost ophthalmic formulation, under clinical development, compared with commercially available BAK-latanoprost and 0.02% BAK, which is the concentration of BAK used in the commercial solution of BAK-latanoprost.

Materials and Methods

Tissue Model and Treatments

The 3D-human corneal epithelial (HCE) model was supplied by SkinEthic Laboratories (Nice, France). It consists of immortalized HCE cells vertically grown on an inert permeable polycarbonate filter measuring 0.5 cm^2 and cultivated for 5 days at the air–liquid interface in a supplemented chemically defined medium (modified MCDB 153; SkinEthic Laboratories). At reception, the 3D-HCEs were equilibrated for 4 hours in the defined maintenance medium supplied, before experiments were done. Thirty microliters of PBS, BAK 0.02%, BAK-latanoprost (Xalatan; Pfizer, New York, NY), and PF-latanoprost (Monoprost; Laboratoires Théa, Clermont-Ferrand, France) were applied on the apical surface of 3D-HCEs for 24 hours and 24 hours followed by 24 hour additional recovery time (Table). The post incubation time was chosen to allow the cells to recover if toxic effects were still reversible. This treatment procedure was expected to model the defense capacity of the tissue better. The apical surface was rinsed twice with 150 μL of PBS after treatment. Two series of 3D-HCEs were subjected to MTT testing at 24 hours and one series was used for inclusion in optimal cutting temperature medium (OCT) followed by immunofluorescence analyses of apoptosis on frozen sections at 24 hours and 24 hours plus 24 hours.

MTT Test

Cellular viability was evaluated in duplicate at 24 hours with the MTT assay[17–19] we had previously modified to improve the sensitivity of the test.[15] The 3D-HCEs were transferred to 24-well plates containing 300 μL of MTT solution diluted at 0.5 mg/mL in the culture medium, and 300 μL of the same MTT solution was applied on the apical surface of the 3D-HCEs. Reconstituted tissues were incubated for 3 hours at 37°C. Then they were transferred to 24-well plates containing 750-μL isopropanol, and 750-μL isopropanol was added to the apical surface of the 3D-HCEs. The plates were agitated for 2 hours at room temperature before reading the optical density (OD) at 570 nm versus OD 690 nm. The results were expressed as percentages of cell viability (mean ± SD) compared with the negative control (PBS).

Animals and Eye Drop Treatments

Twenty four adult male New Zealand albino rabbits weighing 2.5 to 3.0 kg were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Before all experiments, the rabbits were all anesthetized by subcutaneous injection of a 1:5 mixture of 100 mg/mL xylazine (Bayer, Puteau, France) and 100 mg/mL ketamine hydrogen chloride (Imalgène 500; Merial, Lyon, France). Fifty microliters of the test solutions were instilled in both eyes of the rabbits using a micropipette at 5 minute intervals a total of 15 times[16] (Table). The animals were divided into four groups of six rabbits each: five rabbits were used for clinical and IVCM observations at 4 hours (H4) and days (D1) after the administration of the treatment; and the remaining rabbit was killed for immunohistologic procedures at D1. H4 and D1 corresponded to the time course of inflammatory acute phase; H4 showing a significant inflammatory cell infiltration as hyperreflective points observed using HRT and D1 point corresponded to the time we observed the highest rate of inflammatory CD45-positive cells.[5,20]

Clinical Findings and Draize Test

The time was noted when obvious redness occurred on the ocular surface, calculated from the first instillation. The eyes were examined by slit lamp biomicroscopy and were scored according to a modified Draize test.[20]

IVCM Evaluation

The Heidelberg Retina Tomograph II/Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany) laser scanning IVCM was used to examine the rabbits. IVCM scores were used to evaluate ocular surface toxicity profiles in four histologic zones.[20]

Cresyl Violet Staining in CIC

CIC specimens were collected as described in previous studies: two nitrocellulose membranes, 0.2-μL porosity (Millipore, Bedford, MA), were applied to the superior bulbar conjunctiva for the further cresyl violet cytology (1%, number 5235; Merck, Fontenay-sous-Bois, France) procedure. We evaluated cellular damage and inflammatory cell infiltration according to a published CIC score system.[21]

Immunofluorescence Analysis on Frozen Sections of 3D Epithelia and Rabbit Eyes

The 3D construct was transferred into a Petri dish containing 500 μL of PBS and was cut into two pieces under the binocular microscope (Nikon SMZ645; Nikon Instruments, Champigny sur Marne, France). Both pieces of tissue were fixed and embedded in OCT (Tissue-Tek; Miles Inc., Elkhart, IN) and frozen at −80°C. Vertical sections (10-μm thick) were cut with a cryotome (Leica CM 3050s; Leica Microsystems AG, Wetzlar, Germany), and then subjected to TUNEL for the detection of apoptotic cells (Roche Diagnostics, Meylan, France).

Cryosections of enucleated eyes at D1 were prepared and incubated with anti-CD45 (1:50, CBL1412; Cymbus Biotechnology, Chandlers Ford, UK) to detect inflammatory cell infiltration. Apoptotic and inflammatory cells were counted under a fluorescent microscope (Olympus BX-UCB; Olympus, Melville, NY) equipped with a DP70 Olympus digital camera (Olympus). The results were expressed as the mean number of cells per field under the ×400 magnification and mean number of cells per square millimeter, respectively.

Table. Test Items and Controls Used in This Study

Name	Active Ingredient	Batch Number	Preservation System
PBS	None	Not applicable	None
0.02% BAK solution - dilution of 0.1% BAK in PBS	None	FAB 07/05/2009	BAK
Monoprost eye drops	Latanoprost 0.005%	S879	None
Xalatan eye drops	Latanoprost 0.005%	R08267	0.02% BAK

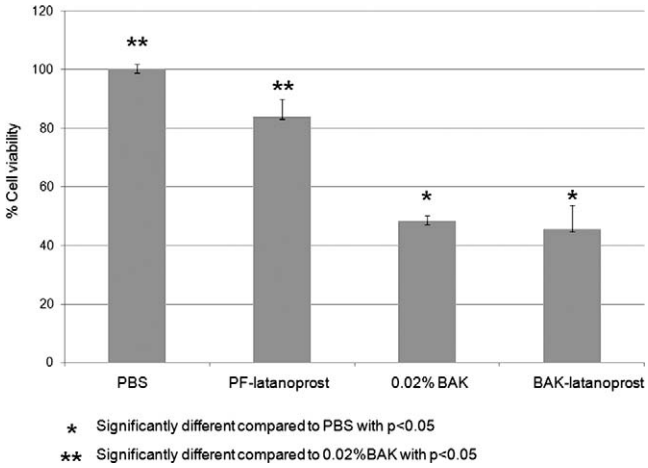

Figure 1. Cellular viability of 3D-HCEs cultures for 24 hours in PBS, PF-latanoprost, 0.02% BAK, and BAK-latanoprost.

Statistical Analysis

Statistical comparisons of control and treatment groups were performed using 2-way ANOVA, followed, if necessary, by multiple pairwise comparisons using Bonferroni adjustment for MTT data and quantification of TUNEL-positive cells and using the Fisher method for pair-wise comparisons for all in vivo data (Statview V; SAS Institute Inc., Cary, NC).

Results

Modified MTT Test

After 24 hours of treatment, BAK and BAK-latanoprost induced a significant 48% and 45% decrease in cellular viability, respectively, as assessed by the MTT ($P < 0.05$ compared with control) (Fig. 1). PF-latanoprost slightly reduced the cell viability to 83%, without reaching significance compared with control. The PF-latanoprost–induced level of viability was significantly higher compared with those induced by BAK and BAK-latanoprost treatments ($P < 0.05$).

Immunofluorescence Analysis and Quantification on Frozen Sections

Rare, homogenously disseminated, TUNEL-positive cells were found in the control 3D-HCE cultures at 24 hours (Fig. 2A) and at 24 hours plus 24 hours (Fig. 2B). BAK-containing solutions significantly increased the number of TUNEL-positive cells, with 44 cells per field and 43 cells per field found at 24 hours (Figs. 2A, 3) and 61 cells per field and 68 cells per field (Figs. 2B, 3) counted at 24 hours plus 24 hours after 0.02% BAK and BAK-latanoprost treatments, respectively. At 24 hours PF-

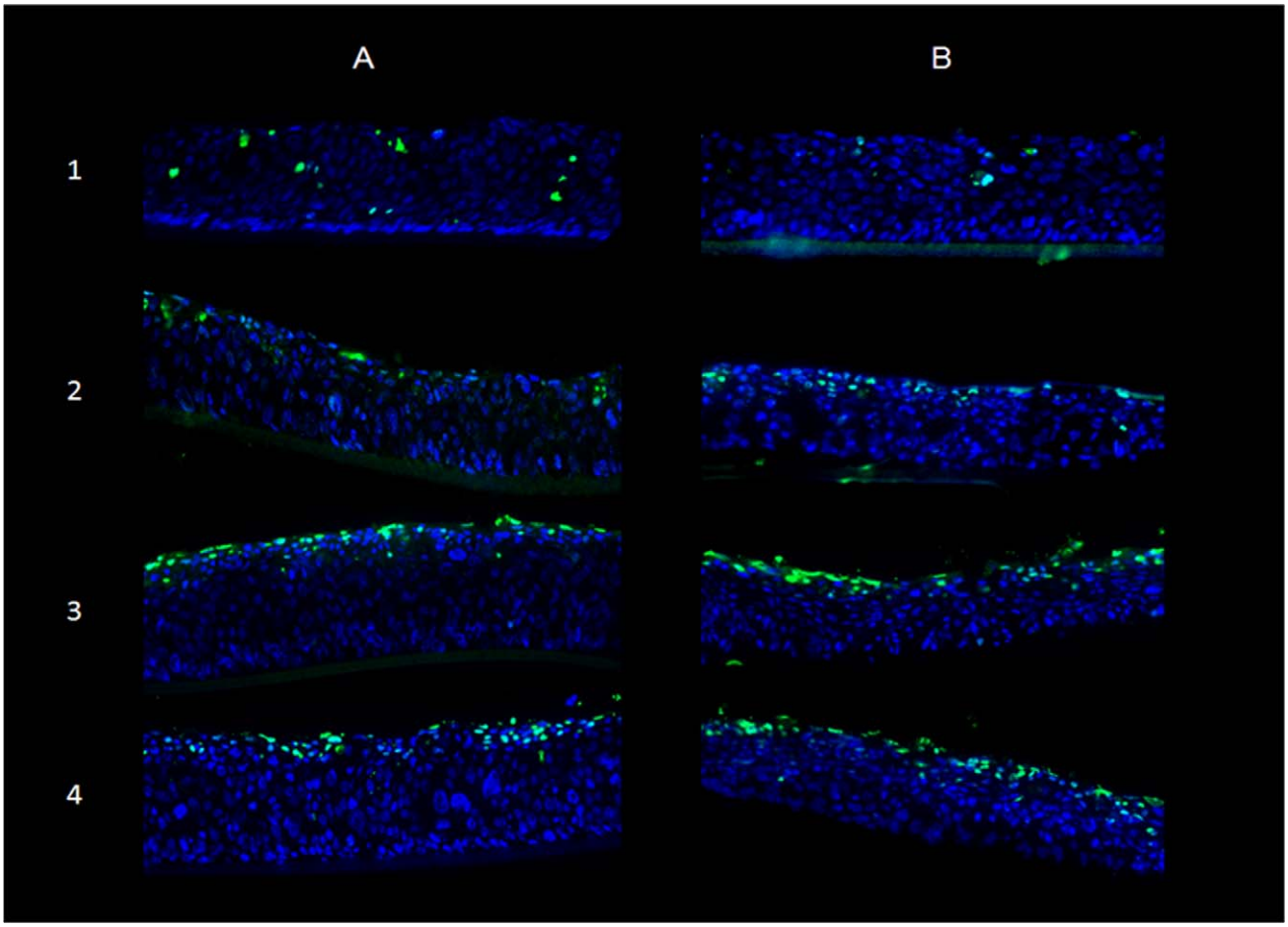

Figure 2. Immunolocalization of TUNEL-positive cells on 3D-HCE cultures treated with (1) PBS, (2) PF-latanoprost, (3) 0.02% BAK, and (4) BAK-latanoprost for (**A**) 24 hours and (**B**) 24 hours followed by a 24 hour post incubation period of time (×200 magnification).

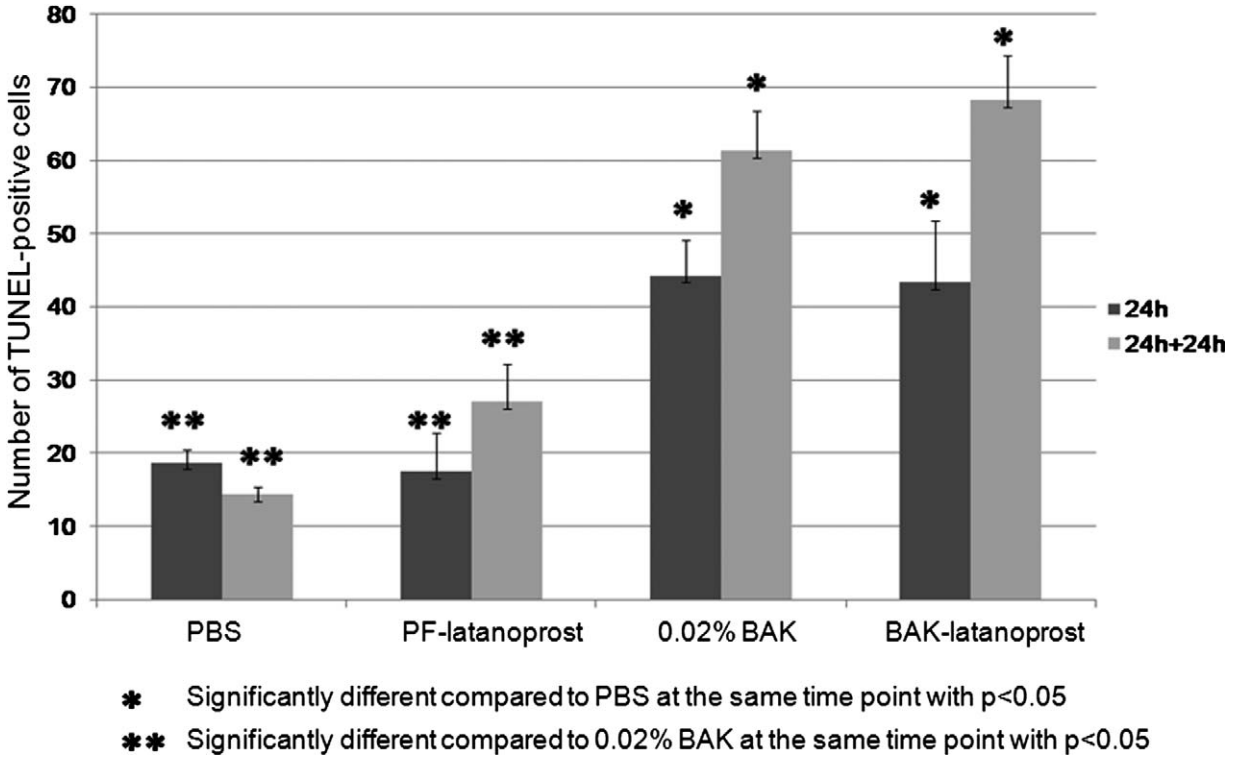

Figure 3. Quantification of TUNEL-positive cells after 24 hour of treatment with PBS, PF-latanoprost, 0.02% BAK, and BAK-latanoprost, followed or not by a 24 hour post incubation period of time.

latanoprost induced an effect that was comparable to that of PBS (Figs. 2A, 3). After the post incubation period, it slightly increased the number of apoptotic cells to 27 cells per field (Figs. 2B, 3), without reaching significance compared with control. Moreover, these effects were considered significantly different from those induced by 0.02% BAK and BAK-latanoprost ($P < 0.05$).

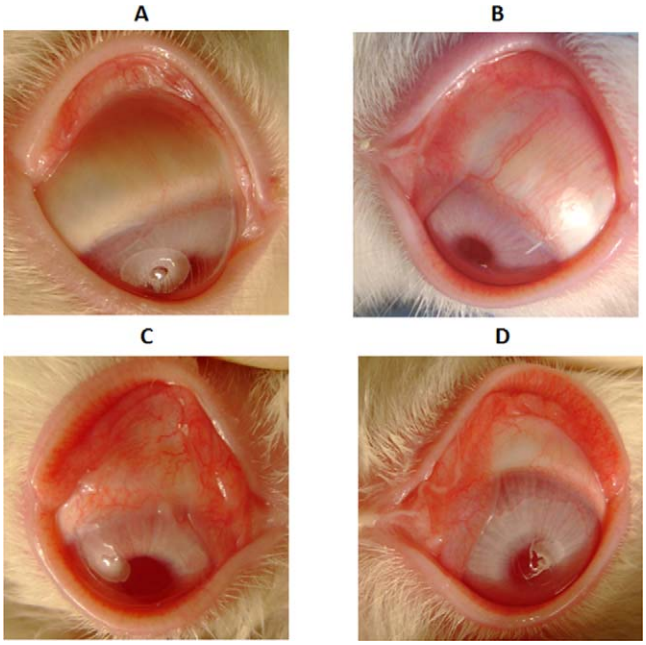

Figure 4. Photographs of rabbit eyes 4 hours after the 15 instillations of (**A**) PBS, (**B**) PF-latanoprost, (**C**) 0.02% BAK, and (**D**) BAK-latanoprost.

Animal Clinical Findings

The PBS group (Fig. 4A) presented the same aspects as found in normal rabbit eyes without obvious abnormality. PF-latanoprost induced diffuse hyperemia in rabbits at H4 (Fig. 4B). Nevertheless, these clinical signs were limited compared with those observed after treatment with 0.02% BAK (Fig. 4C) and BAK-latanoprost (Fig. 4D). In these groups, diffuse hyperemia, chemosis, and secretions on the conjunctiva were observed, with conjunctival redness appearing very quickly after the first instillation. PF-latanoprost presented a slight and late redness.

The PF-latanoprost group showed no statistical difference in ocular Draize scores compared with the PBS group (Fig. 5). At H4, 0.02% BAK and BAK-latanoprost presented higher Draize scores than PBS ($P < 0.0001$ for both) and PF-latanoprost ($P = 0.0007$ and $P = 0.0008$, respectively). At D1, the BAK-latanoprost group returned to a normal aspect, without statistical differences compared with the PBS or PF-latanoprost groups. However, ocular changes were still observed at D1 for BAK with higher scores than for PBS ($P = 0.0257$) and PF-latanoprost ($P = 0.0350$).

IVCM Images and IVCM Score

Superficial Epithelium. At H4 after instillation, PBS- and PF-latanoprost–instilled rabbits presented almost normal aspects of the corneal epithelium, with regular polygonal mosaic appearance, brightly reflective nuclei and no obvious desquamation or swelling (Fig. 6A). Preserved latanoprost and BAK solutions induced various pathological aspects of the corneal epithelium including partial desquamation of epithelial cells,

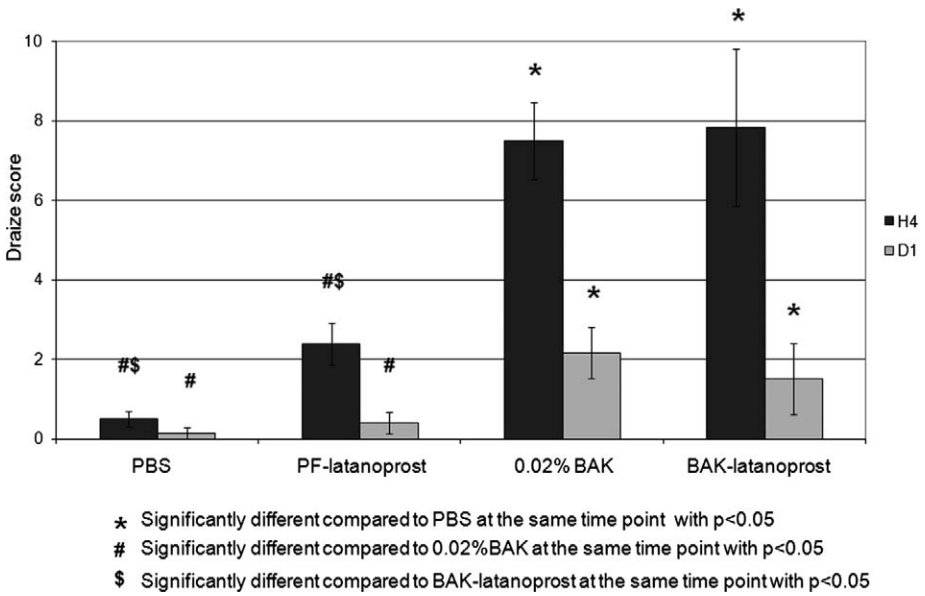

Figure 5. Draize test scores at H4 and D1 after instillations of PBS, PF-latanoprost, 0.02% BAK, and BAK-latanoprost, for 24 hours.

irregular cell shapes, anisocytosis and loss of cell borders, abnormal reflectivity patterns, swollen cells, and inflammatory infiltration (Fig. 6A).

Basal Epithelium. PBS (Fig. 6B) did not induce any inflammation, while PF-latanoprost induced a slight infiltration of cells at H4 only with 23 ± 4.4 cells/mm^2 (Fig. 6B), which was not considered significant compared with PBS. At H4, BAK 0.02% (Fig. 6B) induced inflammation with 79 ± 7 cells/mm^2 and BAK-latanoprost (Fig. 6B) induced an increased inflammatory cell infiltration at significant levels (142 ± 28 cells/mm^2; $P = 0.0002$ compared with PBS and $P = 0.0004$ compared with PF-latanoprost).

Anterior Stroma. We observed a slight inflammatory infiltration and disorganization only after instillations of BAK (Fig. 6C). No abnormalities of the posterior stroma or endothelium were observed with any treatment (data not shown).

Limbus. No obvious responses were seen in the PBS group (Fig. 6D), but in the PF-latanoprost group, sporadic cells were observed in the limbus area at H4 (Fig. 6D). In the 0.02% BAK (Fig. 6D) and BAK-latanoprost (Fig. 6D) groups, we observed obvious inflammatory infiltrations in the peripheral cornea and the limbus area.

Conjunctival Stroma. Both the PBS- and PF-latanoprost–instilled rabbits presented normal conjunctival blood vessels without any rolling inflammatory cells (Fig. 6E). In the 0.02% BAK (Fig. 6E) and BAK-latanoprost (Fig. 2D) groups, obvious rolling of inflammatory cells was recorded.

IVCM score evaluation used to quantify abnormalities over the whole ocular surface showed no significant differences at

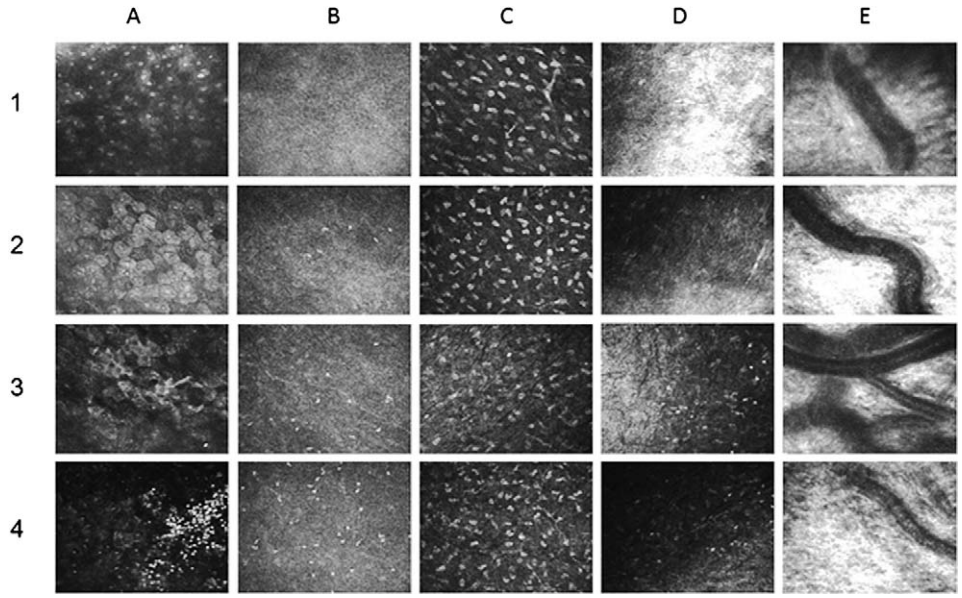

Figure 6. In vivo confocal images of rabbit corneal superficial epithelium (Raw [**A**]), basal epithelium (Raw [**B**]), anterior stroma (Raw [**C**]), limbus (Raw [**D**]), and conjunctival substancia propria (Raw [**E**]) after instillations of (1) PBS, (2) preservative-free PF-latanoprost, (3) 0.02% BAK, and (4) BAK-latanoprost at H4. Photograph size: 400 μm by 400 μm.

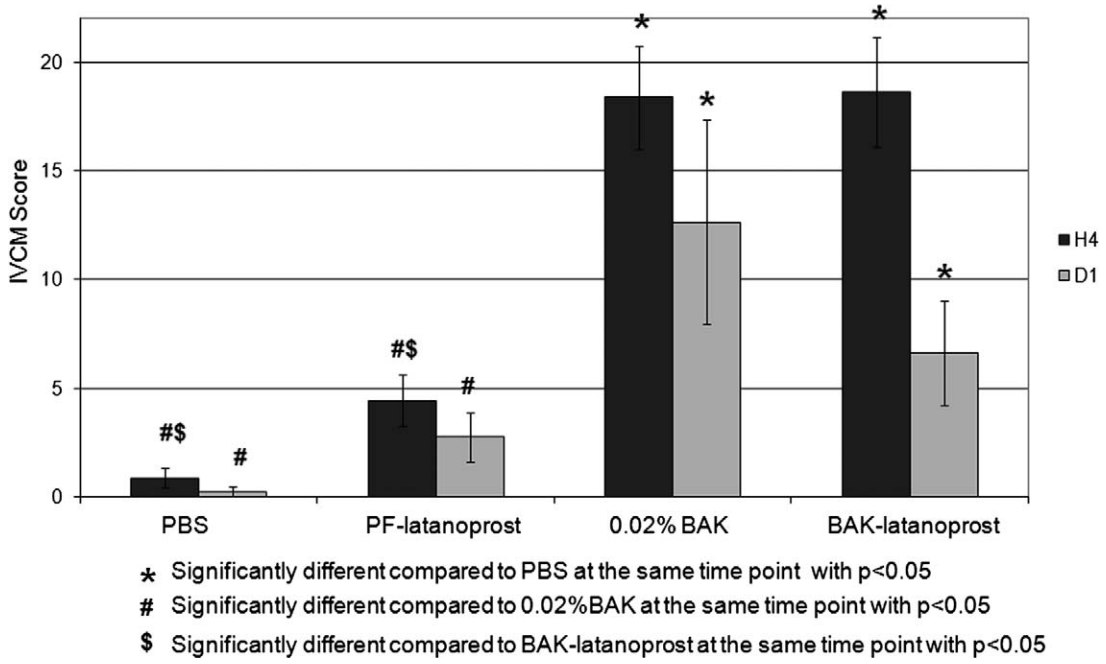

Figure 7. In vivo confocal microscopy scores at H4 and D1 after instillations of PBS, PF-latanoprost, 0.02% BAK, and BAK-latanoprost.

H4 and D1 between PF-latanoprost and PBS (Fig. 7). BAK 0.02% and BAK-latanoprost showed higher IVCM scores compared with both PBS and PF-latanoprost ($P < 0.0001$ for all comparisons) at H4. At D1, IVCM scores were the highest after application of BAK alone ($P < 0.03$ compared with the three other groups), while BAK-latanoprost scores remained higher than the PBS and PF-latanoprost groups, without reaching significance.

Cresyl Violet Staining on CIC

CIC specimens from rabbit eyes instilled with PBS showed a homogeneous cell sheet with a low mean CIC score of 0.75 ± 0.25 and 0.33 ± 0.33 at H4 and D1, respectively (Figs. 8A, 9): the epithelial cells were flat and regular, with a nucleocytoplasmic ratio ranging from 1:2 to 1:3. Goblet cells were clearly visible among or beside the epithelial cells. PF-latanoprost (Figs. 8B, 9) instillation induced a low, but increased CIC score (S = 6.25 ± 2.46) due to slight anisocytosis in the epithelium with normal nuclei and slight infiltration of inflammatory cells. Goblet cell number and morphology remained normal. Important inflammatory infiltration was observed after the instillation of 0.02% BAK (Figs. 8C, 9) and BAK-latanoprost (Figs. 8D, 9) leading to significantly increased CIC scores at H4 (S = 14 ± 1.8 and S = 12.4 ± 2.06, respectively, with $P < 0.0001$ compared with both PBS and PF-latanoprost treatments). In BAK-latanoprost–receiving eyes, a few conjunctival epithelial cells could still be observed, adjacent to inflammatory patches, with substantial anisocytosis and anisonucleosis. In BAK-receiving eyes, patches of inflammatory cell-containing secretions without any epithelial cells were observed. The number of goblet cells dramatically decreased in these conditions.

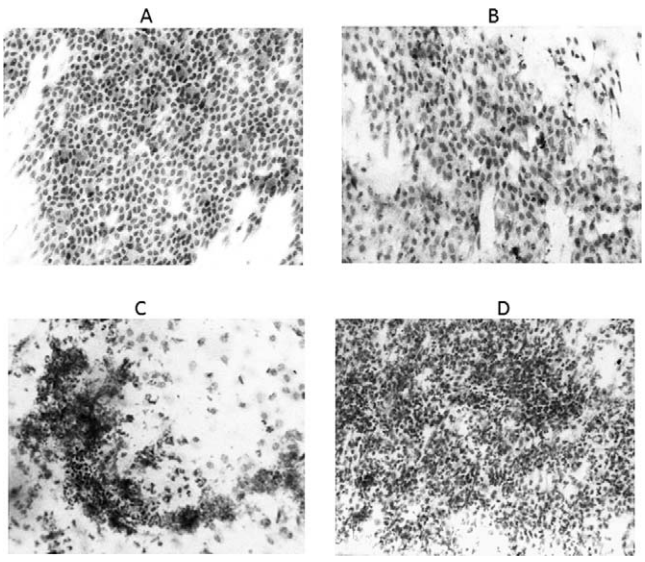

Figure 8. Cresyl violet-stained conjunctival impression cytology from rabbits instilled with (**A**) PBS, (**B**) PF-latanoprost, (**C**) 0.02% BAK, and (**D**) BAK-latanoprost at H4 (×400 magnification).

Immunohistology of CD45-Positive Cells in Rabbit Cryosections

PF-latanoprost–instilled rabbit eyes showed no infiltration of CD45+ inflammatory cells in the peripheral cornea (Fig. 10A) but a slight infiltration in the limbus (675 ± 217 cells/mm^2; Figs. 10B, 11) and conjunctiva (650 ± 87 cells/mm^2; Figs. 10C, 11), with no statistically significant differences with the PBS eye (Figs. 10, 11). BAK 0.02% and BAK-latanoprost induced numerous CD45+ inflammatory cells in the peripheral cornea, limbal, and conjunctival areas: in the limbus 1250 ± 232 cells/mm^2 were found for 0.02% BAK (Figs. 10B, 11); and 1925 ± 368 cells/mm^2 for BAK-latanoprost (Figs. 10B, 11), with no differences. In the conjunctiva, 1425 ± 322 cells/mm^2 were counted for 0.02% BAK (Figs. 10C, 11), and 1525 ± 242 cells/mm^2 for BAK-latanoprost (Figs. 10C, 11). The numbers of CD45+ cells were similar between BAK-latanoprost and BAK,

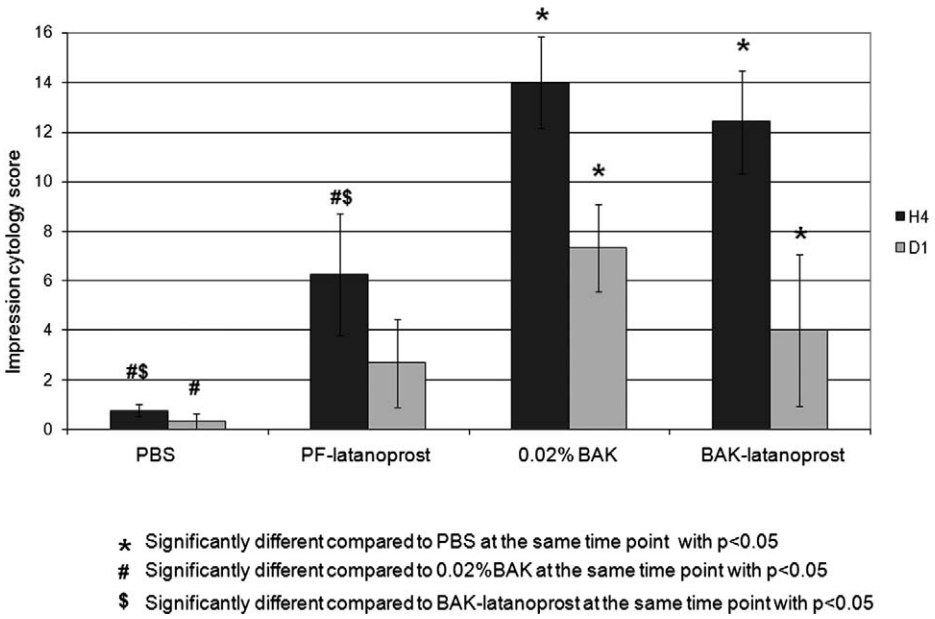

Figure 9. Conjunctival impression cytology scores at H4 and D1.

and were significantly higher in both groups compared with the PBS ($P < 0.0001$) and PF-latanoprost ($P < 0.0001$) groups.

Discussion

Given its similar structure to the living HCE,[22] the 3D-HCE was thought to be a good surrogate of in vivo testing in the field of ocular irritation and ocular safety assessment. It is considered a quality controlled and easy to perform test system, with a high level of reproducibility.[23–25] In a previous study, we optimized the MTT test, adapted to 3D models,[15] so as to improve the sensitivity of the test by taking into account the toxic effects not only in the basal layer, but also in the most superficial layers. With this new procedure, we demonstrated that 0.02% BAK and BAK-latanoprost decreased cell viability at levels close to 50%, while PF-latanoprost slightly decreased cell viability to 83%, with no significant difference compared with PBS, but a significant difference with 0.02% BAK and BAK-latanoprost. Moreover, this result was correlated with the quantification of the apoptotic, TUNEL-positive cells on epithelial tissue cryosections at 24 hours and at 24 hours plus 24 hours, and this study showed that PF-latanoprost induced relatively few apoptotic cells in the superficial layer of the epithelium. On the contrary, 0.02% BAK and BAK-latanoprost induced higher levels of apoptosis in the apical layers of the epithelium, with a significant effect compared with PBS and PF-latanoprost treatments.

We used the well established in vivo toxicological model of acute instillations, which is a good indicator of further absence of ocular toxicity in a more conventional use over the long term for glaucoma patients.[16–20] Using this model, the clinical Draize score of PF-latanoprost was slightly increased, although it did not reach significance. Compared with 0.02% BAK and BAK-latanoprost, which quickly induced redness and swelling, PF-latanoprost seemed to be better tolerated, with limited hyperemia observed at H4 that disappeared at D1.

These results were confirmed at the cellular level, using high-resolution IVCM to follow the morphological changes in the animals' cornea, limbus, and conjunctiva, with an outstanding level of sensitivity. Using the scoring scale previously published by our group, we were able to attribute a score to each product tested that corresponded to the IVCM analysis of the whole ocular surface at a microscopic level.[20] These scores were the highest with 0.02% BAK and BAK-latanoprost treatments at H4, while PF-latanoprost induced a mild score that was not considered significantly different from that of PBS. In particular, only a few inflammatory cells were detected in the basal epithelium and/or in the limbal area at H4, while 0.02% BAK and BAK-latanoprost induced marked inflammation in the same areas. All IVCM scores decreased at D1, with the 0.02% BAK alone score remaining significantly higher compared with PBS. The cytological analysis of conjunctival imprints revealed that PF-latanoprost was the best-tolerated treatment among PF-latanoprost, 0.02% BAK and

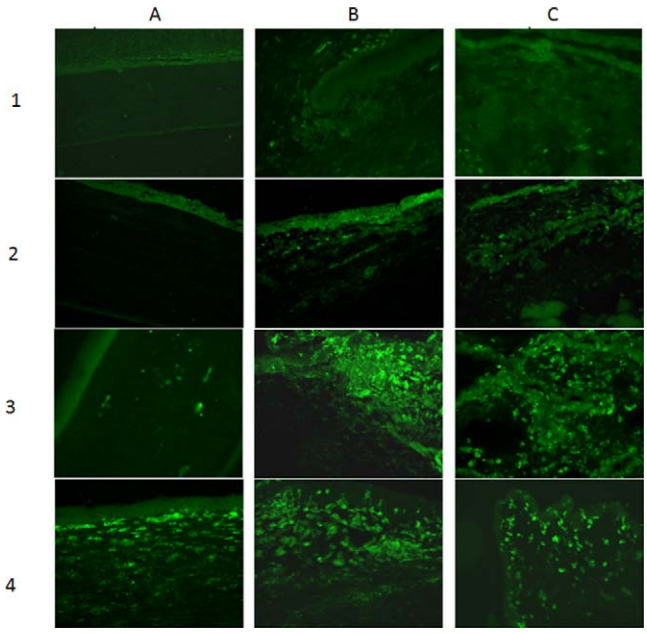

Figure 10. Immunohistology of CD45+ cells (*green*) in (**A**) peripheral cornea, (**B**) limbus, and (**C**) conjunctiva at D1 after instillations of (1) PBS, (2) PF-latanoprost, (3) 0.02% BAK, and (4) BAK-latanoprost.

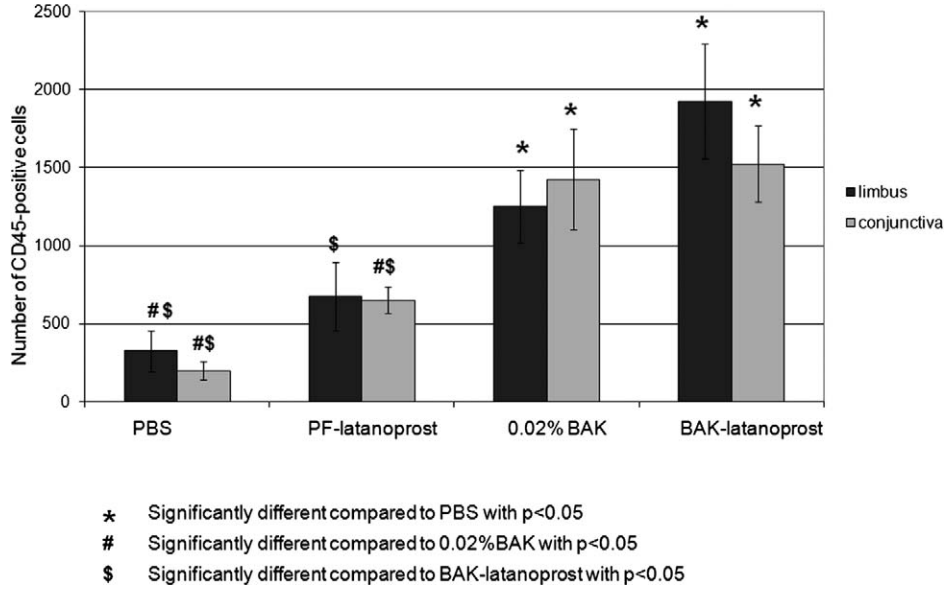

Figure 11. Cell counts of CD45+ cells in limbus and conjunctiva of rabbit cryosections at D1 after treatment with PBS, PF-latanoprost, 0.02% BAK, and BAK-latanoprost.

BAK-latanoprost, as it only induced slight anisocytosis and rare infiltration of inflammatory cells. The other two solutions induced marked infiltration of inflammatory cells and dramatic destruction of the epithelial conjunctival cells and goblet cells. Nevertheless, the recovery at D1 was remarkable with BAK-latanoprost, with a significantly reduced CIC score. The quantification of CD45+ cells on cryosections showed the highest levels of inflammation with 0.02% BAK and BAK-latanoprost. PF-latanoprost-treated eyes disclosed a moderate increase of CD45+ cells in the conjunctiva and the limbus that was not significant compared to PBS. Finally, we observed that the BAK-latanoprost did not exhibit additive toxic effect of the mild PF-latanoprost and the 0.02% BAK cytotoxicity observed at H4 and D1. These results highlight the possible protective effect of the prostaglandin over the preservative toxicity already reported by some studies.[25]

These results are in accordance with many other studies that demonstrated the pro-inflammatory and pro-apoptotic effect of BAK on ocular surface epithelia, beginning with Swan et al. in 1944.[26] Despite the number of articles supporting the deleterious effect of BAK on the ocular surface,[3,5–7] debate continues on the benefit of BAK in eye drop formulations. The first argument advanced to maintain BAK arises from its capacity to increase corneal permeability, acting as a penetration enhancer for active compounds improving their pharmacologic effects.[27–29] In contrast, in a prospective clinical study Gross et al. demonstrated that BAK-free travoprost presents similar IOP-lowering efficacy as BAK-containing travoprost.[30] Furthermore, a comparative pharmacokinetic study did not report any significant difference in the tafluprost concentration found in rabbit aqueous humor between the BAK-associated and BAK-free formulations.[31] Furthermore, a clinical study showed a similar IOP-lowering effect with both formulations of latanoprost (Rouland J, et al. *IOVS* 2012;A240:ARVO E-Abstract 5099). These previous studies suggested that BAK is not necessary to provide drug penetration.[27–31]

Glaucoma is a chronic degenerative pathology that requires permanent pharmacological management. The current treatments are not efficient enough to stop the progress of the disease. More than 50% of glaucomatous patients require the addition of more than one drug, increasing ocular surface BAK accumulation and, thus, the risk of side effect occurrence. Moreover, after many years of treatments, patients could undergo surgery when IOP becomes unresponsive to eye drop medications; therefore, the quality of the ocular surface is an important factor of efficient filtration surgery.[32] The new glaucoma eye drops favor the patient's compliance and, thus, potentiate treatment efficiency by alleviating progressive side effects. Non preserved latanoprost has proved to be better tolerated than BAK-containing formulations and may therefore improve the therapeutic observance and quality of life of glaucoma patients.

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