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Impression cytology of the conjunctival epithelium after antiglaucomatous treatment with latanoprost

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PURPOSE. *To study nongoblet and goblet epithelial conjunctival cells after several treatment periods with latanoprost, a prostaglandin analogue.*
METHODS. *Twelve patients (20 eyes) were studied before the onset of treatment and after 1, 3, and 6 months of latanoprost use. Impression cytology was carried out to analyze cellular density and morphologic parameters such as minimum and maximum diameter and area.*
RESULTS. *Nongoblet epithelium cell density did not change over the treatment period. The density of goblet cells increased after 1 month of use, but returned to initial cell density after longer treatment periods. Nongoblet epithelial cells underwent a significant reduction in size after 1, 3, and 6 months of treatment. In addition, the minimum/maximum diameter ratio suggested that after 1 month there were some changes in shape (a slight elongation) when compared to cells of untreated patients. Nevertheless, after longer treatment periods, the cells regained their original shape. No changes in size were observed in goblet cells, except for a slight decrease in maximum diameter after 6 months of treatment, which suggests that the cells became more rounded.*
CONCLUSIONS. *The density of nongoblet epithelial cells does not change after different treatment periods with latanoprost. However, their size decreases and after short treatment periods their shape also undergoes changes. The density of goblet cells increases after 1 month of treatment, but decreases again after longer periods. Their size does not undergo any modification, although there is a variation in shape after 6 months of treatment. (Eur J Ophthalmol 2003; 13: 553-9)*

KEY WORDS. *Conjunctive epithelium, Nongoblet epithelial cell, Goblet epithelial cell, Impression cytology*

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INTRODUCTION

Glaucoma, a disease affecting more than 50 million people worldwide, has become one of the most common causes of blindness. After more than a century with hardly any changes in treatment methods, several revolutionary therapies that act upon some of the factors involved in the genesis and development of glaucoma have recently been marketed for reducing intraocular pressure (IOP).

These new therapies, which are more efficient and safer for the patient, together with less traumatic and better surgical procedures, have helped to overcome the apparent lack of progress in glaucoma studies.

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Among these new therapies, hypotensive drugs (nonselective beta-blockers, alpha-2 adrenergic agonists, and prostaglandins) are the first choice (1-3). Prostaglandins in particular are able to decrease IOP very efficiently in 20 to 30% of patients (4, 5), but there is little research regarding their effect over long treatment periods (6, 7). Latanoprost (a prostaglandin $F_{2\alpha}$ analogue) is an important drug in the treatment of glaucoma (8). Many patients respond very well to this therapy, but as it has only recently been introduced into the market, information regarding side effects is limited and its long-term effects are unknown (9). Latanoprost presents very good systemic tolerance, although it has been associated with uveitis and cystoid macular edema, and thus is not indicated for postoperative use (10, 11).

In order to determine the effect of topical treatment on the conjunctiva, we carried out impression cytology on the conjunctiva. This is a safe, relatively simple, and painless method of obtaining specimens from the conjunctival surface. It is a valuable diagnostic tool for the early stages of the disease because it is noninvasive and can be used over longer periods (12, 13). In addition, it allows us to analyze the morphology of the conjunctival–corneal surface and the characteristics and density of nongoblet and goblet epithelial cells (14, 15). On the other hand, the comparison between conjunctival biopsy findings and impression cytology confirms that the latter provides the same information as a biopsy (16).

METHODS

Subjects

Our study involved 12 patients (20 eyes) who received antiglaucomatous treatment by instillation of one drop of latanoprost (50 μg/ml, Xalatan; Pharmacia & Upjohn, Uppsala, Sweden) every 24 hours. These patients were not receiving any other topical ocular treatment.

Procedure

A first impression cytology was carried out in each patient before starting the treatment (untreated patients). In the same patients, another sample was taken after 1, 3, and 6 months of treatment. The samples were collected on Millipore HAWP 304 filter paper (Bedford, MA), which was cut into small rectangles (5 x 3 mm) and marked in a corner in order to guarantee correct orientation. One drop of 0.5% proparacaine was instilled at the bottom of the conjunctival sac of the patients as topical anesthetic. Next, the filter paper was applied on the lower bulbar conjunctiva (6 hours) exerting light pressure for at least 3 seconds. The Millipore paper was then pressed onto a microscope slide for the sample to adhere to its surface, and subsequently the paper was removed. One drop of 96% ethanol was placed on the sample for 10 minutes to act as fixative and then the sample was stained using periodic acid–Schiff–hematoxylin. The samples were first hydrated in decreasing concentrations of ethanol, then oxidized in 0.5% periodic acid (10 min.), rinsed in distilled water, stained with Schiff reagent (20 min.), soaked in 0.5% sodium bisulfite (two or three rinses), and then counterstained with Carazzi hematoxylin (5 min.). The specimens were then dehydrated (96° to 100° ethanol) and immersed in xylene. Finally, they were mounted with Entellan (Merck, Darmstadt, Germany) and coverslipped. This staining technique has the advantage of clearly showing the nuclei and the cytoplasm of the nongoblet epithelial cells, thus easily differentiating them from goblet cells. This facilitates cell counting and cell size measurement (Fig. 1).

The parameters under study were evaluated by image analysis, using a RCK black and white camera fitted to a Nikon Labophot optical microscope and a computer with dedicated image analysis software (Visilog 5.2). This analysis allowed us to count both cell populations in the conjunctival epithelium located within our reference area (in our case 0.0052 mm^2), thus obtaining cell density (n/mm^2), and also to obtain some morphometric parameters such as area and maximum and minimum diameter, which provided us with data regarding the morphology of both types of cells.

Statistical analysis

Data are expressed as mean ± SEM. We used the SPSS software package to perform a Student's *t*-test for cell density and a nonparametric Kruskal-Wallis test for the remaining parameters. Similarly, a multiple comparison test was carried out with the mean

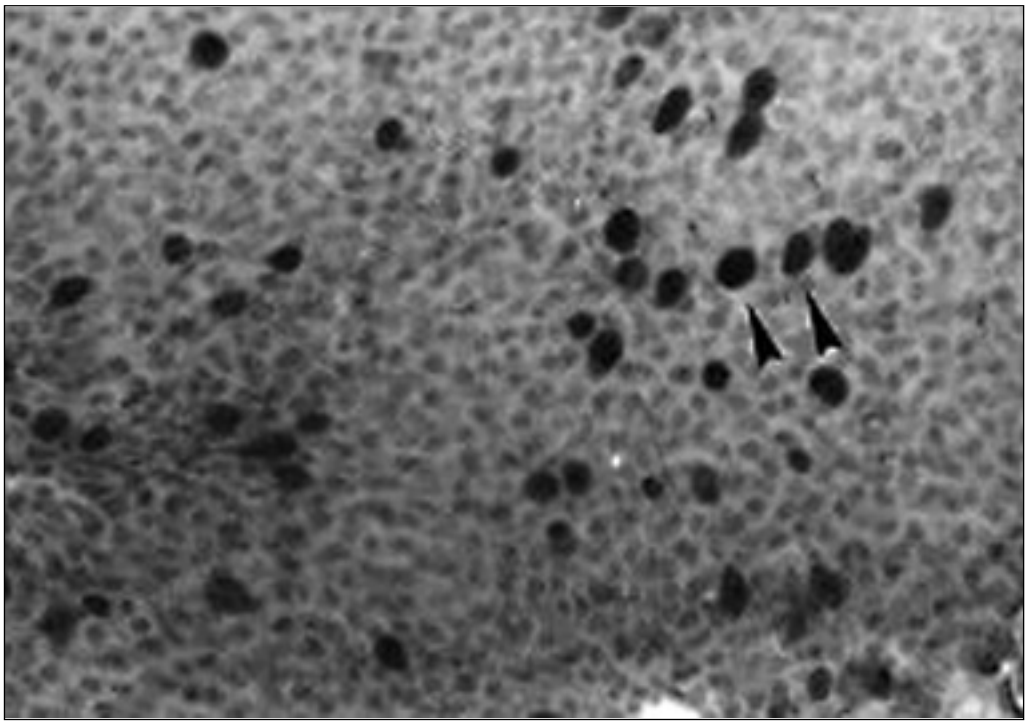

Fig. 1 - *Conjunctival impression cytology (periodic acid–Schiff–hematoxylin staining; original magnification x200). Normal conjunctival epithelium where highly grouped nongoblet cells can be observed, in greater quantities than goblet cells (arrowhead). These cells appear highly stained and larger.*

ranges obtained with the nonparametric Kruskal-Wallis test. A p value less than 0.05 was considered statistically significant.

RESULTS

Nongoblet epithelial cells

Regarding the density of nongoblet epithelial cells, we found 25,673 ± 1,288 cells/mm^2 in untreated patients. After 1, 3, and 6 months of therapy, the number increased, but the differences are not statistically significant (Fig. 2).

Regarding the morphometric parameters under study, the surface in untreated patients was 101.5 ± 1.47 μm^2. After 1, 3, and 6 months of latanoprost therapy, there was a significant decrease compared to untreated patients ($p<0.0001$; Tab. I, Fig. 2). In untreated patients, maximum and minimum cell diameter analysis yielded values of 15.8 ± 0.10 μm and 9.9 ± 0.09 μm, respectively. A significant reduction was observed in both values ($p<0.0001$) after the different treatment periods, which in the case of maximum diameter took place after the third month (Tab. I, Fig. 2).

From the maximum and minimum diameter values we obtained the shape of the cells, given by the quotient. The adimensional values obtained ranged from 0 to 1, where 1 represented a sphere. Thus, in untreated patients, we obtained a value of 0.62 for nongoblet epithelial cells, which underwent a slight elongation after the first month of treatment (0.52). After 3 and 6 months of therapy, no differences were found in shape when compared to untreated patients.

Goblet epithelial cells

In untreated patients, we observed a cell density of 942 ± 61 cells/mm^2 (Tab. II, Fig. 3). After 1 month of treatment with latanoprost, this value increased significantly to 1,340 ± 119 ($p<0.0005$; Tab. II, Fig. 3), and although it dropped after 3 and 6 months of treatment, no significant differences were found.

Regarding morphometric parameters, the cell surface in untreated patients was 193.4 ± 3.34 μm^2, with a nonsignificant decline after the three treatment periods (Tab. II, Fig. 3). The maximum diameter dropped significantly ($p<0.01$) after 6 months of treatment, but

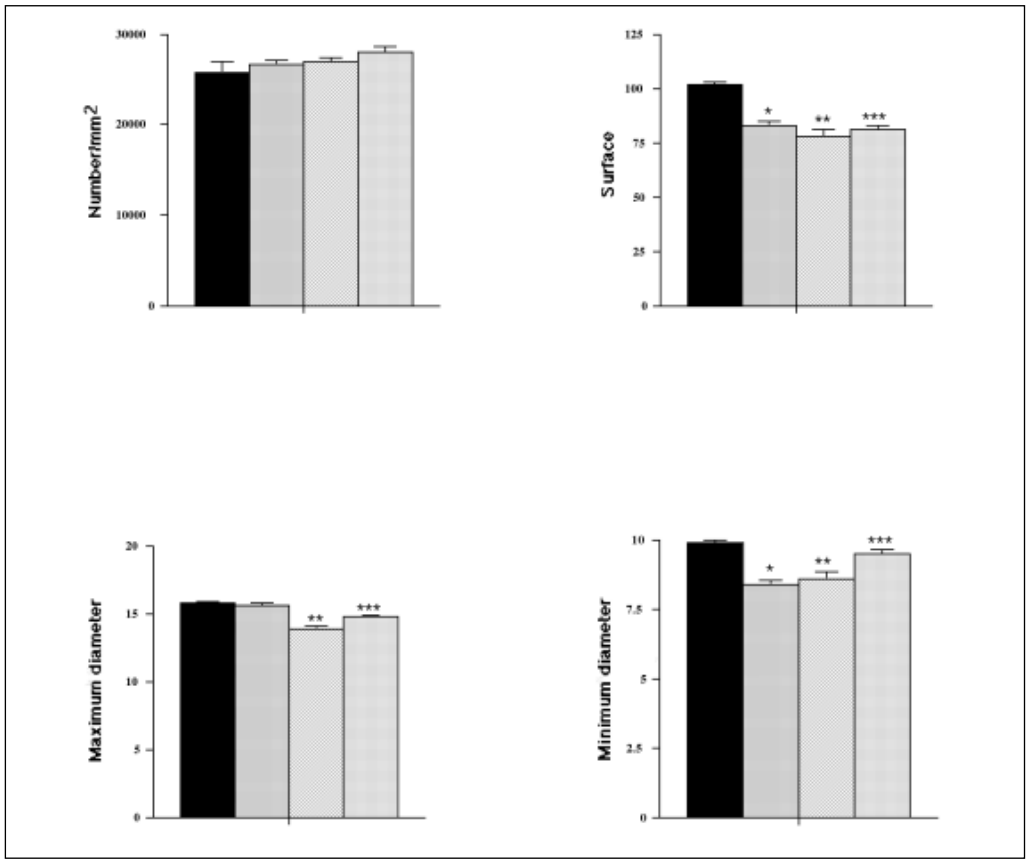

Fig. 2 - *Nongoblet cells. Different parameters corresponding to untreated patients (black-filled box), and after 1, 3, and 6 months of treatment with latanoprost (noncontinuous line, wide line, and dot-filled boxes, respectively). Statistical significance *between 1 month of treatment and untreated patients; **between 3 months of treatment and untreated patients; and ***between 6 months of treatment and untreated patients.*

TABLE I - DATA CORRESPONDING TO NONGOBLET EPITHELIAL CONJUNCTIVAL CELLS

Group	Number/mm^2	Surface, μm^2	Maximum diameter, μm^2	Minimum diameter, μm^2
Untreated	25,673 ± 1,288	101.5 ± 1.47	15.8 ± 0.10	9.9 ± 0.09
After 1 month of treatment	26,634 ± 480	82.6 ± 1.88	15.6 ± 0.16	8.4 ± 0.13
After 3 months of treatment	26,980 ± 442	77.7 ± 3.05	13.9 ± 0.19	8.6 ± 0.23
After 6 months of treatment	27,903 ± 692	81.2 ± 1.37	14.8 ± 0.10	9.5 ± 0.13

the minimum diameter did not undergo any change (Tab. II, Fig. 3).

The shape of the goblet cells in untreated patients was given by a ratio of 0.77, and no changes were found until 6 months of therapy, when the cells became slightly rounder (0.81).

DISCUSSION

We investigated the degree of squamous metaplasia occurring in patients treated with latanoprost for glaucoma after different treatment periods. The degree of squamous metaplasia provides us with infor-

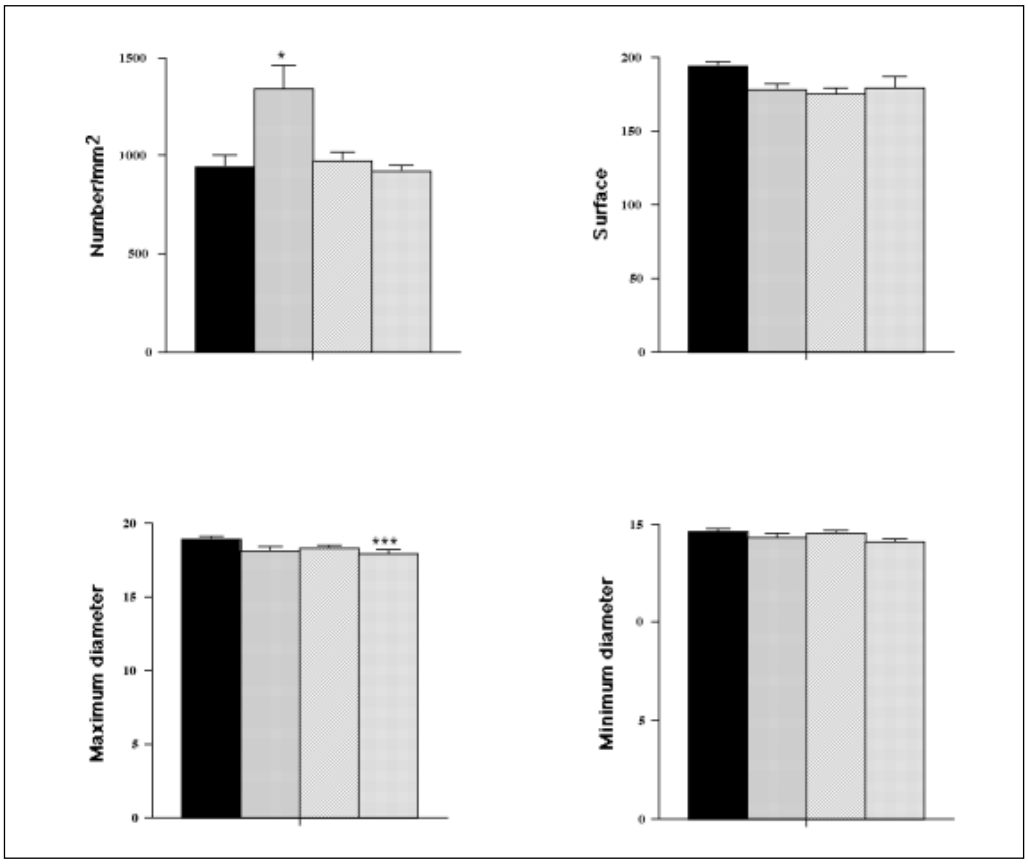

Fig. 3 - *Goblet cells. Different parameters corresponding to untreated patients (black-filled box), and after 1, 3, and 6 months of treatment with latanoprost (noncontinuous line, wide line, and dot-filled boxes, respectively). Statistical significance *between 1 month of treatment and untreated patients, and ***between 6 months of treatment and untreated patients.*

TABLE II - DATA CORRESPONDING TO GOBLET EPITHELIAL CONJUNCTIVAL CELLS

Group	Number/mm^2	Surface, μm^2	Maximum diameter, μm^2	Minimum diameter, μm^2
Untreated	942 ± 61	193.4 ± 3.34	18.9 ± 0.15	14.6 ± 0.16
After 1 month of treatment	1,340 ± 119	177.8 ± 4.10	18.1 ± 0.22	14.3 ± 0.2
After 3 months of treatment	975 ± 44	174.4 ± 4.6	18.3 ± 0.15	14.5 ± 0.18
After 6 months of treatment	917 ± 34	178.6 ± 3.25	17.9 ± 0.25	14.1 ± 0.14

mation about the state of the ocular surface, as this is directly related to the severity of the metaplasia (17, 18). In more specific terms, the study of goblet epithelial cells is highly relevant because a loss or a decrease in their density is an early sign of squamous metaplasia (15, 19). We analyzed the two cell populations present in the conjunctival epithelium—i.e., goblet and nongoblet epithelial cells—and calculated their numbers per surface unit (cell density) as well as several morphometric parameters that provided us with data on cellular morphology. The alterations in these cells following the instillation of antiglaucomatous drugs

is a controversial issue and we therefore aimed to provide further data that could help us better understand this ocular pathology as well as its treatment.

Regarding cellular density, untreated patients presented values of 25,673 ± 1,288 nongoblet cells/mm^2 and 942 ± 61 goblet cells/mm^2, thus revealing that almost 95% of the cell population corresponds to nongoblet epithelial cells. The density ratio between nongoblet and goblet cells did not change after the different therapy periods, although there was a significant increase in goblet cell density after 1 month of treatment. Nevertheless, the ratio between both populations remained at 95 to 96%/4 to 5% throughout the treatment period, suggesting that latanoprost therapy does not modify the ratio found in untreated patients.

Most clinical data found in the literature regarding the use of latanoprost deal with IOP measurements (20-22), and the only study providing data that can be compared to our own is that of Mietz et al (23), who reported that the number of goblet cells in albino rabbits treated with latanoprost and timolol decreased after 18 months of treatment. However, our study did not cover such a long period.

The analysis of the area in both cell populations showed a significant decrease in the size of nongoblet epithelial cells after 1, 3, and 6 months of treatment (18.62%, 23%, and 20%, respectively, compared to untreated patients). In other words, the decline is stronger after the longer treatment periods (3 and 6 months). These findings confirm that latanoprost has an iatrogenic effect on the nongoblet epithelial cells of the conjunctiva and that there is a decrease in their size after the first month of treatment that persists 6 months after treatment. However, these changes in size do not initially correlate with a loss of nongoblet epithelial cells. According to our data, the size of goblet epithelial cells declined nonsignificantly after the three periods of treatment under study (8%, 9.82%, and 7.65%). When the cellular area of both cell populations in untreated patients was compared, we found that the goblet cells were larger than the nongoblet epithelial cells (+47.5%). This difference increased in treated patients, where goblet cells were around 54% larger than nongoblet epithelial cells, probably owing to the significant reduction in size of the latter.

The diameter study yielded a similar pattern to the one described for nongoblet epithelial cells. However, the fact that the maximum diameter did not change after 1 month of therapy might suggest that cells became slightly elongated, but returned to their initial shape after 3 and 6 months of treatment. In goblet cells, the maximum diameter declined only after 6 months of therapy, whereas the minimum diameter did not present significant differences between treated and untreated patients. This suggests that, as confirmed by the shape factor, after 6 months of treatment goblet cells adopt a rounded shape, although their size is not altered.

The mechanisms involved in this chain of events during the use of latanoprost are unknown. The iatrogenic effect of the preservatives used in eyedrops on the nongoblet and goblet epithelial cells of the conjunctiva is well known. Latanoprost could give rise to a certain degree of histotoxicity and a decrease in cellular size, which could lead to atrophy, and ultimately to cellular death with the consequent decrease in the number of cells. However, the absence of important modifications in goblet epithelial cells of the conjunctiva leads us to think that in patients treated with latanoprost these types of cells are less vulnerable than nongoblet epithelial cells. The persistence of the values obtained for goblet cells suggests a normal secretory conjunctival mucosa and, therefore, no squamous metaplasia should be expected in the periods under study. Further studies on the clinical effects of latanoprost involving longer treatment periods would be useful, given that short-term changes appear to be insignificant.

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REFERENCES

1. Le Jeume C, Munera Y, Hughes FC. Systemic effects of three β_1-blocker eyedrops: comparison in healthy volunteers of β_1 and β_2-adrenoreceptor inhibition. Clin Pharmacol Ther 1990; 47: 578-83.
2. Brooks AMV, Gillies WE. Ocular β_1-blockers in glaucoma management. Clinical pharmacology aspects. Drugs Aging 1992; 2: 208-21.
3. Strahlman ER, Vogel R, Tipping R, Clineschmidt CM. The use of dorzolamide and pilocarpine as adjunctive therapy to timolol in patients with elevated intraocular pressure. The Dorzolamide Additivity Study Group. Ophthalmology 1996; 103: 1283-92.
4. Stewart WC, Holmes KT, Mark A. Washout periods for brimonidine 0.2% and latanoprost 0.005%. Am J Ophthalmol 2001; 131: 798-9.
5. Toris CB, Zhan GL, Zhao J, Camras CB, Yablonski ME. Potential mechanism for the additivity of pilocarpine and latanoprost. Am J Ophthalmol 2001; 131: 722-8.
6. Garcia Sanchez J, Garcia Feijó J. Mantenimiento médico del glaucoma. Arch Soc Esp Oftalmol 2000; 75: 11-20.
7. Nuzzi R, Emanuelli L, Fiazzo C. Evaluation of conjunctival tearing functionality and impression cytology in glaucomatous patients using long-term-medication. New Trends Ophthalmol 1996; 11: 41-6.
8. Camras CB, Alm A, Watson P, Stjernschantz J. Latanoprost. A prostaglandin analog for glaucoma therapy. Ophthalmology 1996; 103: 1916-24.
9. Stewart WC, Sharpe E, Stewart JA, Holmes KT, Latham KE. Additive efficacy of unoprostone isopropyl 0.12% to latanoprost 0.005%. Am J Ophthalmol 2001; 131: 339-44.
10. Waevar RE, Bullock J, Ballal D. Cystoid macular edema and anterior uveitis associated with latanoprost used. Experience and incidence in a retrospective review of 84 patients. Ophthalmology 1998; 105: 263-8.
11. Miyake K, Ota I, Meekbo K, Ichihashi S, Miyake S. Latanoprost accelerates disruption of the blood-aqueous barrier and the incidence of angiographic cystoid macular edema in early postoperative pseudophakias. Arch Ophthalmol 1999; 117: 34-40.
12. Egbert PR, Lauber S, Maurice MD. A simple conjunctival biopsy. Am J Ophthalmol 1977; 84: 798-801.
13. Nelson JD. Impression cytology. Cornea 1988; 7: 71-81.
14. Kinosita S, Kiorpes TC, Friend J, Thoft RA. Goblet cell density in ocular surface diseases. Arch Ophthalmol 1983; 101: 1282-7.
15. Tseng SH, Chen YT, Cheng HC, Huang FC, Lee SC, Chen FK. Impression cytology study of conjunctival epithelial phenotypes on the healing ocular surface after pterygium excision. Cornea 2001; 20: 244-50.
16. Reddy M, Reddy PM, Reddy SC. Conjunctival impression cytology in dry eye states. Ind J Ophthalmol 1991; 39: 22-4.
17. Nelson JD, Wright JC. Conjunctival goblet cell densities. Arch Ophthalmol 1983; 102: 1049-51.
18. Tseng SCG. Staging of conjunctival squamous metaplasia by impression cytology. Ophthalmology 1985; 92: 728-33.
19. Tseng SC, Hirst LW, Maumenee AE, Kenyon KR, Sun TT, Green WR. Possible mechanism for the loss of goblet cells in mucin-deficient disorders. Ophthalmology 1984; 91: 545-52.
20. Widengard I, Maepea O, Alm A. Effects of latanoprost and dipivefrin, alone or combined, on intraocular pressure and on blood-aqueous barrier permeability. Br J Ophthalmol 1998; 82: 404-6.
21. Studer ME, Martin CL, Stiles J. Effects of 0.005% latanoprost solution on intraocular pressure in healthy dogs and cats. Am J Vet Res 2000; 61: 1220-4.
22. DuBiner H, Cooke D, Dirks M, Stewart WC, VanDenburgh AM, Felix C. Efficacy and safety of bimatoprost in patients with elevated intraocular pressure: a 30-day comparison with latanoprost. Surv Ophthalmol 2001; 45: S353-60.
23. Mietz H, Schlötzer-Schrehardt U, Strassfeld C, Krieglstein GK. Effect of latanoprost and timolol on the histopathology of the rabbit conjunctiva. Invest Ophthalmol Vis Sci 2001; 42: 679-87.