DOI: 10.1590/1809-6891v24e-75047E

Ciência Animal Brasileira
Brazilian Animal Science

e-ISSN 1809-6891

Section: Veterinary medicine
Research article

Evaluation of the corneal epithelium of rabbits treated with preservative-free eye drops containing ketorolac tromethamine or diclofenac sodium

Avaliação dos colírios cetorolaco de trometamina e diclofenaco sódico sem conservantes no epitélio da córnea de coelhos

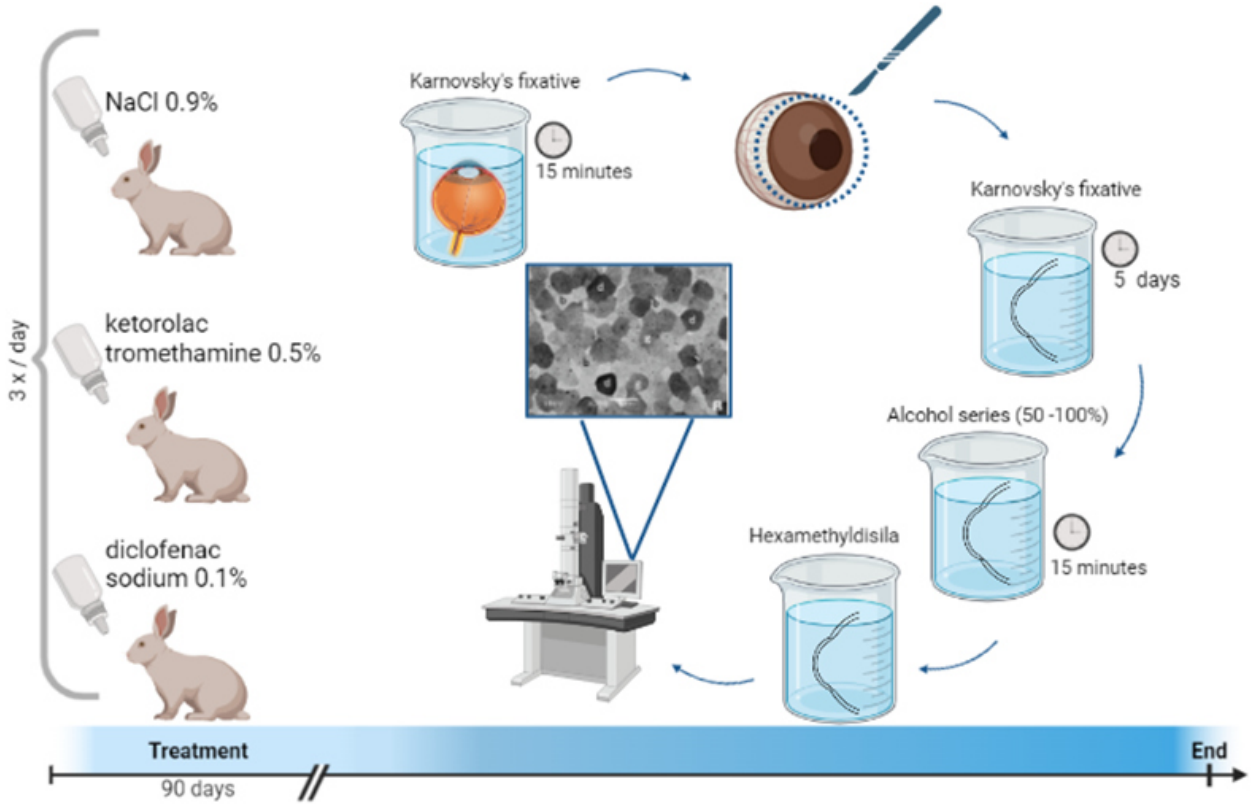
Fabiana Quartiero Pereira[1], Melina Barbara Bender[1], Tanise Carboni da Silva[1], Bruna Santos dos Santos[1], Paula Stieven Hünning[1], Cláudia Skilhan Faganello[1], Maira Haase Pacheco[1*], João Roberto Braga de Mello[1], Fernanda Bastos de Mello[1], João Antonio Tadeu Pigatto[1]

[1]Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil
[*]Corresponding author: mairahaase@gmail.com

Abstract
This study aimed to evaluate the corneal epitheliotoxic effects of preservative-free ketorolac tromethamine 0.5% and diclofenac sodium 0.1% eye drops in rabbits. Seventeen New Zealand rabbits were randomly divided into three groups: the 0.5% ketorolac tromethamine group, the 0.1% diclofenac sodium group, and the control group (0.9% NaCl). For each rabbit, both eyes were treated three times daily according to their treatment group. The corneal epithelia were analyzed using scanning electron microscopy to observe the number of light, grey, and dark cells; the number of epithelial holes; and the loss of hexagonal shape. Both of the formulations administered caused changes in the healthy corneal epithelia of rabbits. Except for number of epithelial holes ($p < 0.05$), all the parameters showed a statistically significant difference between the groups. The number of dark cells was highest in the ketorolac tromethamine group ($p<0.05$). The number of grey cells was higher in the diclofenac sodium group than in the control group ($p =0.003$). A higher number of dark cells was associated with a smaller number of light cells ($r =-0.577$, $p < 0.001$). Loss of shape showed a direct correlation with the number of dark cells ($r=0.524$, $p=0.002$). Based on the results presented, it was possible to conclude that ketorolac tromethamine 0.5% was more toxic to rabbit corneal epithelium than diclofenac sodium 0.1%.
Keywords: cornea; nonsteroidal anti-inflammatory agent; ophthalmic solutions; scanning electron microscopy

Resumo
Objetivou-se avaliar os efeitos do cetorolaco de trometamina a 0,5% e do diclofenaco de sódico a 0,1% sem conservantes na córnea de coelhos. Dezessete coelhos da raça Nova Zelândia foram aleatoriamente divididos em três grupos: o grupo de 0,5% de cetorolaco de trometamina, o grupo de 0,1% de diclofenaco sódico e o grupo controle (0,9% de NaCl). Para cada coelho, os dois olhos foram tratados três vezes ao dia durante 90 dias de acordo com o grupo de tratamento. Os epitélios da córnea foram analisados usando microscopia eletrônica de varredura para observar o número de células claras, cinzas e escuras, o número de criptas e a perda do formato celular hexagonal. Ambas as formulações administradas causaram alterações no epitélio da córnea de coelhos. Com exceção da contagem de criptas ($p <0,05$), todos os parâmetros apresentaram diferença estatisticamente significante entre os grupos. O número de células escuras foi maior no grupo cetorolaco de trometamina ($p <0,05$). O número de células cinzentas foi maior no grupo diclofenaco de sódio do que no grupo controle ($p=0,003$). O maior número de células escuras observado foi associado ao menor número de células claras ($r=-0,577$, $p<0,001$). A perda do formato celular mostrou uma correlação direta com o número de células escuras ($r=0,524$, $p=0,002$). O cetorolaco de trometamina 0,5% foi mais tóxico para o epitélio da córnea de coelhos do que o diclofenaco de sódio a 0,1%.
Palavras-chaves: córnea**;** antiinflamatório não esteroidal; solução oftálmica; microscopia eletrônica de varredura

Received: February 8, 2023. Accepted: April 18, 2023. Published: May 8, 2023.



Graphical abstract - Evaluation of the corneal epithelium of rabbits treated with preservative-free eye drops containing ketorolac tromethamine or diclofenac sodium

1. Introduction

Topical applied non-steroidal anti-inflammatory (NSAIDs) drugs are commonly administered in patients with specific inflammatory ocular conditions and to prevent miosis during cataract surgery [1,2]. Ophthalmic formulations with NSAIDs are regarded as safer alternatives to those containing corticosteroids for prolonged treatment[3,4,5]. However, the corneal epithelium is sensitive to a number of topical medications, and the dose, treatment time, and individual predisposing factors affect the potential for epithelial toxicity [6,7]. Some topical drugs might not only accentuate cell desquamation and increase the number of microprojections, dark cells, and the number of epithelial holes, but also cause intercellular junction changes, leading to an increased space between adjacent cells and the loss of hexagonal shape [3,8].

The toxicity of commercially available NSAID eye solutions against corneal epithelial cells *in vitro* compared with that of steroid eye solutions, with special attention to drug concentration and exposure time was investigated [2]. Rabbits have been employed in the evaluation of the effects of drugs on the corneal epithelium [3,8]. Some studies related the toxicity of commercial NSAID eye solutions on the corneal epithelial healing [2,3,9,10,11]. However, the effects of NSAID eye solution preservative-free on the corneal epithelium of rabbits have not been investigated.

The aim of the present study was to evaluate the corneal epitheliotoxic effects of preservative-free ketorolac tromethamine 0.5% and diclofenac sodium 0.1% eye drops on rabbits using scanning electron microscopy.

2. Material and methods

Seventeen healthy, female, albino, 90-day-old New Zealand rabbits that weighed between 2 and 2.5 kg were studied. Prior to the beginning of the experiment, eye exam on all rabbit was realized. The examination consisted of evaluation with slit-lamp biomicroscopy (Portable Slit Lamp SL 15, Kowa, Japan), fluorescein

stain (Fluorescein, Allergan, SP, Brazil), Schirmer tear test (Ophthalmos, São Paulo, Brazil), lissamine green test (Ophthalmos, São Paulo, Brazil), and applanation tonometry (TonoPen® AVIA, Reichert Technologies, NY, USA). No abnormalities were found on ophthalmic examinations. This study was authorised by the Committee on Ethics in the Use of Animals (CEUA) of the Federal University of Health Sciences of Porto Alegre (UFCSPA) (case number 14-147/2014). The animals were kept at the Federal University of Rio Grande do Sul (UFRGS) Veterinary Clinic Hospital at constant humidity (50–55%) and temperature (21 ± 2°C) with a light/dark cycle of 12 hours (with light from 8:30 a.m. to 8:30 p.m.) in stainless steel cages. They received standardised feed and were given water *ad libitum*. After a seven-day adaptation period, the rabbits were randomly divided into three groups: the control group (n=12), the ketorolac tromethamine group (n=10), and the diclofenac sodium group (n=12). The housing, management, and euthanasia of the animals were in accordance with Law No. 11,794 of 8 October 2008 (Lei Arouca) and Decree No. 6.899 of 15 July 2009, and respected the ethical principles in experimental research determined by the Brazilian College of Animal Experimentation (COBEA) and the euthanasia practice guidelines of the National Animal Experimentation Control Council. The rabbits had both eyes treated with 1 drop of designated ophthalmic formulation three times daily according to the rabbit's group. The ketorolac tromethamine 0.5% (Ophthalmos ind., Pharmaceuticals Ltda, Sao Paulo, Brazil) and diclofenac sodium 0.1% Ophthalmos ind., Pharmaceuticals Ltda, Sao Paulo, Brazil) eye drops were compounded without preservatives. The control group received saline solution (0.9% NaCl). The three groups were evaluated for 90 days. The ocular surfaces of all animals were examined weekly using portable slit lamp biomicroscopy.

After 90 days of treatment, the rabbits were euthanized and samples were prepared to be studied using scanning electron microscopy (SEM). Karnovsky's fixative solution was immediately instilled on the corneal surface. The ocular bulbs were then enucleated and submerged in the Karnovsky's fixative solution for 15 minutes. All corneal layers came into contact with the fixative. The corneas were removed via 360° limbal peritomy, with a corneal-scleral ring formed using a scalpel blade nº 15 approximately 2 mm from the corneal limbus. The samples were kept in the Karnovsky's fixative solution under refrigeration for 5 days to keep the corneal surface completely flat. After preservation, the samples were dehydrated in an ethyl alcohol series (50, 60, 70, 80, 90, and 100%) for 15 minutes at each concentration. Subsequently, the samples were desiccated, with the alcohol gradually replaced with hexamethyldisilazane to avoid shrinkage of the samples during drying. The samples were wrapped in a specimen holder with copper conductive tape and carbon glue, with the epithelial layer facing upwards, and metallised with a 35 nm layer of gold and palladium. Each sample was evaluated using scanning electron microscopy (JSM 6060, JEOL, Tokyo, Japan) operating at 10kV and 15kV. At least three photomicrographs of the central corneal epithelial area were obtained at different magnifications (350 to 1200x). The photomicrographs were blinded for evaluation. At 500× magnification, the photomicrography contained an average of 70 whole cells, which were randomly selected for analysis and manually counted by an experienced operator. Additionally, the following characteristics were observed: number of light, grey, and dark cells; number of epithelial holes; and degree to which cells had lost their hexagonal shape. The loss of hexagonal shape was scored from zero to five using a subjective and semiquantified system in which a score of zero indicated that all cells present in the field had a hexagonal shape and a score of five represented total loss of hexagonal shape. The number of light, dark, and grey cells and the loss of shape were described for each group.

2.1 Statistical analysis

The mean, standard deviation, median, minimum, and maximum were compared between the groups using the Kruskal-Wallis test, with a Dunn's multiple comparison test utilised as required. The whole sample and the sample for each group were evaluated for the number of dark cells compared to the number of other cells, and for the loss of shape using the Spearman's correlation. The tests were performed with a 5% significance level.

3. Results

Throughout the experiment, all corneas in both groups remained normal, as assessed by slit lamp biomicroscopy, and all corneas remained fluorescein negative. With SEM it was possible to obtain images from all analyzed samples (Figures 1-3).

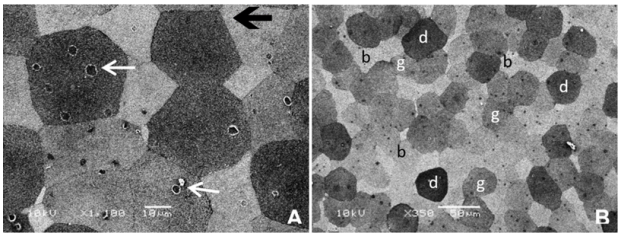


Figure 1. Healthy rabbit corneal epithelium (GC). Figure 1A: Dark cell in hexagonal shape (black arrow) and presence of epithelial hole (white arrow). Figure 1B: light (b) and gray (g) cells are observed, in addition to a small number of dark cells (d).

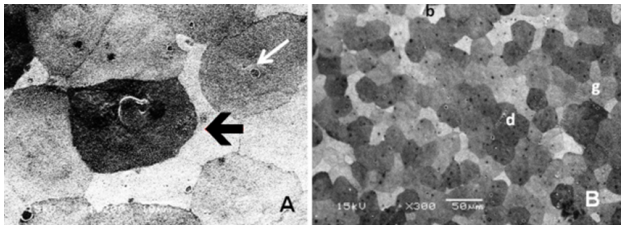


Figure 2. Rabbit corneal epithelium from the diclofenac group. A: Dark cell with a score of 3 for hexagonal shape loss (black arrow) and the presence of epithelial hole (white arrow). B: A number of grey (g) and dark cells (d), resulting in a decrease in the number of light cells (b) compared to the control group, were observed.

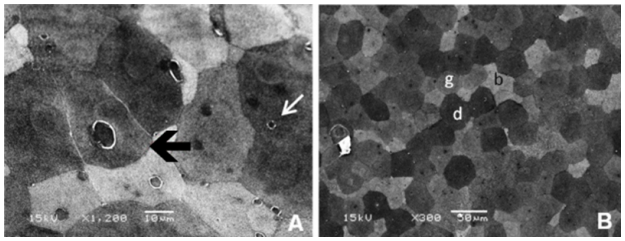


Figure 3. Corneal epithelium of the ketorolac group. A: Dark cell with a score of 4 for the loss the hexagonal shape (black arrow) and the presence of epithelial hole (white arrow). B: There is an increase in the number of grey (g) and dark (d) cells and a decrease in the number of light cells (b) compared to the control group.

Description of appearance of the corneal epithelium of rabbits observed by SEM for each group compared to the control group is summarized in Table 1 and multiple comparisons between groups for cell types and loss of shape is in Table 2.

Table 1. Description of appearance of the corneal epithelium of rabbits observed by SEM for each group. The number of light, dark, and grey cells, number of epithelial holes, and the loss of shape were described (Mean±SD).

Variable	Group			
	Control	Diclofenac Sodium	Ketorolac Tromethamine	p Value
Dark cells	21.2±6	24.2 ±6.3	28.6±3.9*	0.008*
Grey cells	25.5±4.8	32.1±6.8*	29.4±3.5	0.017*
Light cells	23.5±4.1	13.8±3.8*	12±3.6*	<0.001*
Epithelial hole	15.9±12.4	31.4±21.5	21±10.7	0.114
Loss of shape	1.6±0.7	2.8±1.1*	3.7±1.3*	0001*

*Statistically significant values according to a Kruskal-Wallis test ($p<0.05$) compared to the control group.

In the ketorolac tromethamine group, there were significantly more dark cells ($p< 0.05$) cells than in the other groups and fewer light cells and a high degree of loss of hexagonal shape compared to the control groups. The diclofenac sodium group showed significantly more grey cells than the control group, however, there was no difference in the number of dark cells between these two groups. Overall, there was an inverse correlation between the number of dark cells and the number of light cells; a higher number of dark cells was associated with a lower number of light cells ($r=-0.577$, $p<0.001$). In addition, loss of shape was directly correlated with the number of dark cells ($r=0.524$, $p=0.002$). Regarding the number of epithelial holes, there was no statistical difference between the groups studied.

Table 2. Multiple comparisons between groups for cell types and loss of shape

Variable	Comparisons between groups	Z value	P value
Dark cell	Control x Diclofenac Sodium	-1.07	0.283
	Control x Ketorolac Tromethamine*	-3.16	0.002*
	Diclofenac Sodium x Ketorolac Tromethamine*	-2.18	0.029*
Grey Cell	Control x Diclofenac Sodium *	-2.93	0.003*
	Control x Ketorolac Tromethamine	-1.62	0.106
	Diclofenac Sodium x Ketorolac Tromethamine	1.20	0.228
Light cells	Control x Diclofenac Sodium *	3.77	<0.001*
	Control x Ketorolac Tromethamine *	4.24	<0.001*
	Diclofenac Sodium x Ketorolac Tromethamine	0.65	0.513
Loss of shape	Control x Diclofenac Sodium *	-2.27	0.023*
	Control x Ketorolac Tromethamine*	-3.55	<0.001*
	Diclofenac Sodium x Ketorolac Tromethamine	-1.41	0.160

*Statistically significant values according to a Dunn's multiple comparison test ($p<0.05$)

4. Discussion

Topical NSAIDs have become widely accepted for controlling ocular inflammation [11,12,13], nevertheless, this experiment have shown that use of commercial topical NSAIDs may induce some degree of cell damage to ocular tissue, accordingly with previous studies [9,10,14,15,16,17,18,19]. Ophthalmic solutions contain preservatives to maintain the shelf life of the product and to prevent contamination during the treatment period of the patient [11] and these preservatives used can also cause ocular surface toxicity [1,9,20,21]. A study evaluating the effect of six commercially NSAIDs eye drops on the normal corneal epithelium of rabbits using SEM was conducted by Stroobants et al.[3]. All test compounds caused alterations and damage to the cells of the corneal epithelium. The authors also concluded that cell damage appeared to be related to the active ingredient in the eye drops, particularly the type of preservative used [3]. Owing to that fact, it is extremely important to evaluate the isolated pharmacological effect, therefore, research on the use of eye drops without preservatives reduces biases and promotes more robust results in relation to the tested

formulations.

In the present study, the effect of both eye drops on the healthy corneal epithelium of rabbits was compared using SEM. SEM has been used to observe the quantitative and qualitative morphological characteristics of the most superficial cells of the corneal epithelium [8,22].

In the current study, the general appearance of the corneal epithelium of the control group agrees with that described in previous studies [3,8]. In particular, at 500× magnification, the tissue showed an uninterrupted cell mosaic with different grey tonalities, shapes, and sizes. The three classic groups of epithelial cells were observed (light, grey, and dark cells). These different cell sizes and shapes have already been reported and appear to be related to the phenomenon of constant epithelial renewal [8,22]. Cell nuclei were only evident in an extremely small number of cells. Almost all of the cells were covered by a high number of epithelial holes, and there was a considerable variety of shapes among different cells [8,22].

Normal epithelial cells have a hexagonal form and affected epithelial cells tend to lose the hexagonal pattern [3]. In the present study, the cellular pattern was altered by both NSAIDs. Diclofenac sodium caused a larger increase in the number of grey cells, and ketorolac tromethamine induced a larger increase in the number of dark cells. The observed positive correlation between the loss of hexagonal shape and increase in the number of dark cells is consistent with the results obtained by Stroobants et al.[3]. Even in healthy epithelial, it is possible to visualize cells with different characteristics, because in the process of renewal, the surface cells tend to peel. However, with exaggerated cell desquamation, the barrier function of the epithelium may be compromised, allowing the passage of substances from the external environment to the stroma, resulting in stromal changes [8,22]. Indeed, an increased presence of dark cells has been related to a higher degree of cellular exfoliation [8].

Ketorolac tromethamine with benzalkonium chloride preservative might delay the regeneration of the corneal epithelium [15]. The preservatives contained in eye drops can damage the epithelial surface of the cornea. Benzalkonium chloride is the most commonly used eye drop preservative and has been associated with toxic effects such as “dry eye” [9]. Recent studies have demonstrated that benzalkonium chloride inhibits the mitochondria of human corneal epithelial cells [3]. Since the eye drops used in the present study were free of preservatives, we can infer that the loss of hexagonal shape and the increase in the number of dark cells observed in both experimental groups were due to the toxicity of the tested anti-inflammatories. There was no statistically significant difference between ketorolac tromethamine and diclofenac sodium regarding the loss of hexagonal shape or the number of epithelial holes. By Stroobants et al.[3] identified an increase in the number of epithelial holes in the corneal epithelia of rabbits treated with Voltaren® eye drops; this increase was also observed with other commercial formulations of different NSAIDs. Given that, these formulations used different preservatives, the authors attributed this increase to the low pH of the vehicles.

Visual observation is an adequate approach if the only goal is to identify cells with a high or low proportion of the surface covered by surface projections. However, to detect early epithelial changes, it is necessary to measure the epithelial holes density, the average size of microprojections, and the area of the cell surface covered by microprojections. Additionally, a visual assessment of microprojection characteristics is a difficult task, even for an expert, given the complexity of microprojection arrangements [8].

5. Conclusion

The findings of this study enabled us to conclude that the NSAIDs ketorolac tromethamine 0.5% and diclofenac sodium 0.1% cause changes in the corneal epithelium of rabbits. Ketorolac tromethamine 0.5% was more toxic than diclofenac sodium 0.1% to the corneal epithelium of rabbits.

Conflicts of interest

None of the authors have any potential conflicts of interest to disclose.

Author Contributions

Conceptualization: F. Q. Pereira, J. R. B.de Mello, J. A. T. Pigatto and F. B. de Mello. *Methodology:* F. Q. Pereira, P. S. Hünning, C. S. Faganello, B. S. dos Santos, M. B. B. Macedo, J. R. B.de Mello, J. A. T. Pigatto and F. B. de Mello. *Investigation*: F. Q. Pereira, B. S. dos Santos, M. H. Pacheco, T. C. da Silva, J. R. B.de Mello, J. A. T. Pigatto and F. B. de Mello. *Resources:* J. R. B.de Mello and J. A. T. Pigatto. *Writing (original draft, review & editing)*: F. Q. Pereira, B. S. dos Santos, M. H. Pacheco, T. C. da Silva, J. R. B.de Mello and J. A. T. Pigatto.

Acknowledgments

We thank Microanalysis and Microscopy Center (CME/UFRGS) for support for realization of this research.

References

1. Flach AJ. Corneal melts associated with topically applied nonsteroidal anti-inflammmatory drugs. Transactions of the American Ophthalmological Society. 2001;(99):205-2. (https://pubmed.ncbi.nlm.nih.gov/11797308/)

2. Lee JS, Kim YH, Park YM. The Toxicity of nonsteroidal anti-inflammatory eye drops against human corneal epithelial cells in vitro Journal of Korean medical science. 2015;30(12):1856-64. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4689832/)

3. Stroobants A, Fabre K, Maudgal PC. Effect of non-steroidal anti-inflammatory drugs (NSAID) on the rabbit corneal epithelium studied by scanning electron microscopy. Bulletin de la Societe belge d'ophtalmologie. 2000;276:73-82. (https://www.ophthalmologia.be/download.php?dof_id=65)

4. Guidera AC, Luchs JI, Udell IJ. Keratitis, ulceration, and perforation associated with topical nonsteroidal anti-inflammatory drugs. Ophthalmology. 2001;108(5):936-44. (https://www.aaojournal.org/article/S0161-6420(00)00538-8/fulltext)

5. Kim SJ. Flach AJ, Jampol LM. Nonsteroidal anti-inflammatory drugs in ophthalmology. Survey of ophthalmology 2010;55(2):108-33. (https://pubmed.ncbi.nlm.nih.gov/20159228/)

6. Shimazaki J, Saito H, Yang HY. Persistent epithelial defect following penetrating keratoplasty: an adverse effect of diclofenac eyedrops. Cornea. 1995;14(6):623-7. (https://journals.lww.com/corneajrnl/Abstract/1995/11000/Persistent_Epithelial_Defect_Following_Penetrating.18.aspx)

7. Ayaki M, Iwasawa A, Soda M, Yaguchi S, Koide R. Cytotoxicity of five fluoroquinolone and two nonsteroidal anti-inflammatory benzalkonium chloride-free ophthalmic solutions in four corneoconjunctival cell lines. Clinical ophthalmology. 2010;20(4):1019-24. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2946991/)

8. Ferraz LCB, Schellini AS, Gregorio EA. Efeito do colírio de 5-fluorouracil sobre o epitélio corneano íntegro de coelhos. Arquivos Brasileiros de Oftalmologia 2003;66(4):493-7. (https://www.scielo.br/j/abo/a/HXxmrgbppG4vchsdTPdwNDx/?lang=pt)

9. Epstein SP, Ahdoot M, Marcus E, Asbell AP. Comparative toxicity of preservatives on immortalized corneal and conjunctival epithelial cells. Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics. 2009;25(2):113-9. (https://pubmed.ncbi.nlm.nih.gov/19284328/)

10.Hashemian MN, Rahimi F, Alipour F, Ahmadraji AA, Torabi HR, Tavakoli N, Moghadam RS. The effect of topical diclofenac sodium 0.1% on the corneal epithelial healing after photorefractive keratectomy. Iranian Journal of Ophthalmology. 2011;23(2):27-30. (https://www.sid.ir/paper/599363/en)

11.Kawahara A, Utsunomiya T, Kato Y, Takayanagi Y. Comparison of effect of nepafenac and diclofenac ophthalmic solutions on cornea, tear film, and ocular surface after cataract surgery: the results of a randomized trial. Clinical ophthalmology. 2016;4(10):385-91. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4786065/)

12.Aragona P, Tripodi G, Spinella R, Laganá E, Ferreri G. The effects of the topical administration of non-steroidal anti-inflammatory drugs on corneal epithelium and corneal sensitivity in normal subjects. Eye. 2000;(14):206-10. (https://pubmed.ncbi.nlm.nih.gov/10845018/)

13.Duan P, Liu Y, Li J. The comparative efficacy and safety of topical non-steroidal anti-inflammatory drugs for the treatment of anterior chamber inflammation after cataract surgery: a systematic review and network meta-analysis. Graefe's archive for clinical and experimental ophthalmology [Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie]. 2017;255(4): 639-49. (https://pubmed.ncbi.nlm.nih.gov/28130595/)

14.Barba KR, Samy A, Lai C, Perlman JI, Bouchard CS. Effect of topical anti-inflammatory drugs on corneal and limbal wound healing. Journal of cataract and refractive surgery. 2000;26(6):893-7.(https://pubmed.ncbi.nlm.nih.gov/10889437/)

15.Gaynes BI, Onyekwuluje A. Topical ophthalmic NSAIDs: a discussion with focus on nepafenac ophthalmic suspension. Clinical ophthalmology. 2008;2(2):355-68. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2693998/)

16.Wilson DJ, Schutte SM, Abel SR. Comparing the efficacy of ophthalmic NSAIDs in common indications: a literature review to support cost-effective prescribing. The Annals of pharmacotherapy 2015 49(6):727-34. (https://pubmed.ncbi.nlm.nih.gov/25725037/)

17.Iwamoto S, Koga T, Ohba M, Okuno T, Koike M, Murakami A, et al. Non-steroidal anti-inflammatory drug delays corneal wound healing by reducing production of 12- hydroxyheptadecatrienoic acid, a ligand for leukotriene B_4 receptor 2. Scientific Reports. 2017;7(1):13267.(https://www.nature.com/articles/s41598-017-13122-8)

18.Raizman MB, Hamrah P, Holland EJ, Kim T, Mah FS, Rapuano CJ, Ulrich RG. Drug-induced corneal epithelial changes. Survey of ophthalmology. 2017;62(3):286-301. (https://pubmed.ncbi.nlm.nih.gov/27890620/)

19.Rigas B, Huang W, Honkanen R. NSAID-induced corneal melt: Clinical importance, pathogenesis, and risk mitigation. Survey of ophthalmology. 2020;65(1):1-11. (https://pubmed.ncbi.nlm.nih.gov/31306671/)

20.Guo Y, Satpathy M, Wilson G, Srinivas SP. Benzalkonium chloride induces dephosphorylation of myosin light chain in cultured corneal epithelial cells nvestigative ophthalmology & visual science. 2007;48(5):936-44. (https://pubmed.ncbi.nlm.nih.gov/17460253/)

21.Uematsu M, Mohamed YH, Onizuka N , Ueki R , Inoue D , Fujikawa A. Acute corneal toxicity of latanoprost with different preservatives. Cutaneous and ocular toxicology 2015;35(2):1-6. (https://pubmed.ncbi.nlm.nih.gov/26113030/)