Conjunctival inflammation in patients under topical glaucoma treatment with indication to surgery[1]

Inflamação conjuntival em pacientes em tratamento tópico para glaucoma com indicação de cirurgia

João Marcello Furtado[I], Jayter Silva Paula[II], Edson Garcia Soares[III], Neifi Hassam Saloum Dhegaide[IV], Eduardo Melani Rocha[V], Eduardo Donadi[VI], Maria de Lourdes Veronese Rodrigues[VII]

[I]Fellow PhD degree, Postgraduate Program in Ophthalmology, Otorhinolaringology and Head and Neck Surgery, FMRP-USP, Ribeirao Preto-SP, Brazil. Main author. Conception, design, intellectual and scientific content of the study; acquisition, analysis and interpretation of data; manuscript writing, critical revision.
[II]PhD, Assistant Professor, Ophthalmology Division, FMRP-USP, Ribeirao Preto-SP, Brazil. Conception, acquisition and interpretation of data, supervised all phases of the study, manuscript writing and critical revision.
[III]Associate Professor, Pathology Division, FMRP-USP, Ribeirao Preto-SP, Brazil. Conception, analysis and interpretation of data; manuscript writing and critical revision.
[IV]MSc, PhD, Researcher, Laboratory of Molecular Immunology, HCFMRP-USP, Ribeirao Preto-SP, Brazil. Conception of the study, technical support, manuscript writing and critical revision.
[V]PhD, Associate Professor, Ophthalmology Division, FMRP-USP, Ribeirao Preto-SP, Brazil. Supervised all phases of the study, manuscript writing and critical revision.
[VI]PhD, Full Professor, Immunology Division, FMRP-USP, Ribeirao Preto-SP, Brazil. Conception and supervised all phases of the study, manuscript writing and critical revision.
[VII]PhD, Full Professor, Ophthalmology Division, FMRP-USP, Ribeirao Preto-SP, Brazil. Conception, design, intellectual and scientific content of the study; acquisition, analysis and interpretation of data; manuscript writing, critical revision.

ABSTRACT

PURPOSE: To compare the frequency of conjunctival HLA-DR expression (a surrogate marker for inflammation) in eyes treated with topical prostaglandin analogues versus eyes treated with other topical antiglaucomatous drugs.

METHODS: Patients diagnosed with primary open-angle glaucoma presenting indication for trabeculectomy were divided in groups according to the use or not of prostaglandin analogues. All subjects were treated with the maximum tolerated dose of antiglaucomatous drugs until the date of the surgery. At the beginning of the surgical procedure, a 5 x 5 mm biopsy of the bulbar conjunctiva was collected, incubated with monoclonal anti-HLA-DR antibody and processed for histological analysis.

RESULTS: Of the 31 eyes included (31 patients), 25 were under topical prostaglandin analogues (Group 1) and six under other topical pharmacological agents (Group 2). Fourteen eyes of Group 1 (56%) and three of Group 2 (50 %) were positive for the inflammatory marker HLA-DR (P=1.0). The percentage of stained cells ranged from 15.49 to 48.09% (median: 27.61) in Group 1, and from 18.35 to 28 (median: 20.71) in Group 2, with no differences statistically significant (p=0.33).

CONCLUSION: The use of prostaglandin analogues did not increase conjunctival expression of HLA-DR compared to other topical antiglaucomatous agents.

Key words: Leukocyte Antigens. Glaucoma, Open-Angle. Immunology. Ophthalmic Solutions. Prostaglandin analogs.

RESUMO

OBJETIVO: Comparar a frequência da expressão conjuntival de HLA-DR (marcador inflamatório) em olhos tratados com análogos de prostaglandinas de uso tópico com a frequência em olhos tratados com outros medicamentos.

MÉTODOS: Pacientes com glaucoma primário de ângulo aberto apresentando indicação de trabeculectomia foram agrupados segundo o uso ou não de análogos de prostaglandinas. Todos os participantes foram tratados com medicação máxima tolerada até o momento da cirurgia. Ao início do procedimento cirúrgico, uma biópsia de 5 x 5 mm da conjuntiva bulbar foi coletada, incubada com anticorpo monoclonal anti-HLA-DR e processada para análise histológica

RESULTADOS: Dentre os 31 olhos incluídos (31 pacientes), 25 estavam em uso de análogos de prostaglandinas (Grupo 1) e seis em uso de outros agentes antiglaucomatosos (Grupo 2). Quatorze olhos do Grupo 1 (56%) e três do Grupo 2 (50%) apresentaram positividade para o marcador HLA-DR (p=1,0). A porcentagem de células coradas variou de 15,49 a 48,09% (mediana: 27,61%) no Grupo 1 e de 18,35 a 28% (mediana: 20,71%) no Grupo 2, com diferenças não estatisticamente significativas (p=0,33).

CONCLUSÃO: O uso de análogos de prostaglandinas não aumenta a expressão conjuntival de HLA-DR comparado com outros medicamentos tópicos para o tratamento de glaucoma.

Descritores: Antigenos Leucocitários. Glaucoma de Ângulo Aberto. Imunologia. Soluções Oftálmicas. Prostaglandinas Sintéticas.

Introduction

Glaucoma affects up to 60.5 million people worldwide, and more than 8 million of them are blind[1]. Medical therapy is the first choice therapy to reduce intraocular pressure and reduce the risk of visual field loss[2]. Among topical agents, prostaglandin analogues are currently the first-line therapy for glaucoma[3], and some of the side-effects related to their use are iris pigmentation, transient conjunctival hyperemia[4], corneal punctate staining[5], squamous metaplasia[6] and subclinical conjunctival inflammatory reaction that, although subclinical, may be detected by inflammatory markers, such as HLA-DR, expressed on epithelial cells[7-10].

There are studies in the literature describing that chronic topical therapy for glaucoma increases the risk of trabeculectomy failure, and that the preoperative conjunctival inflammation caused by the chronic antiglaucomatous medication would be involved in this process[11]. But although most of the studies report conjunctival inflammation in prostaglandin analogues users[7,8,10,12], this feature may also be present in patients under the use of other antiglaucomatous drugs[9,10,13-15].

Guenoun *et al*.[16] studied, in vitro, conjunctiva-derived cells under the action of prostaglandin analogues in commercial solutions, prostaglandin F2 alpha, TNF-alpha, and of benzalkonium chloride (BAK). The results suggested that toxicity is induced by the preservative rather than the medication itself. Uusitalo *et al*.[17] reported that even patients using preservative-free prostaglandin analog may present conjunctival expression of HLA-DR[17].

As the success of glaucoma surgical treatment depends on the status of the conjunctival and subconjunctival tissue that will be part of the surgical bleb[11], it is important to analyze if prostaglandin analogues are able to induce subclinical inflammation, also if this inflammation is higher than the one caused by other topic agents.

In this study, we selected patients with primary open-angle glaucoma with indication to trabeculectomy surgery, divided them in groups according to the use or not of topical prostaglandin analogues, and compared the conjunctival expression of HLA-DR.

Methods

The investigation was carried out according to Helsinki Declaration (Medical School of Ribeirao Preto University Hospital Ethics Committee protocol number 15582/2005). The expression of the inflammatory marker HLA-DR was analyzed in biopsies of the bulbar conjunctiva from patients diagnosed with primary open-angle glaucoma presenting indication for trabeculectomy, followed at the Glaucoma Outpatient Clinic of the University Hospital, Faculty of Medicine of Ribeirao Preto-University of Sao Paulo.

Subjects were divided in two groups according to the use or not of topic prostaglandin, and exclusion criteria were: presence of pterygium[18], signs of clinical inflammation, and any type of previous surgery in the studied eye. All patients were treated with the maximum tolerated dose of antiglaucomatous drugs until the date of the surgery.

At the beginning of the surgical procedure, a 5 x 5 mm biopsy of the inferior bulbar conjunctiva was collected (6 o'clock position), fixed in formol and processed for histological analysis.

The preparation of the slides has been previously described[12]. Briefly, slides were incubated at room temperature in a moist chamber using the primary monoclonal anti-HLA-DR antibody at a 1:50 concentration (Dako™, Glostrup, Denmark). Histological sections of human lymphoma were used as positive controls to examine the immunologic reaction. Negative controls consisted of the same histological sections but did not include the step of incubation with the primary antibody; this antibody was replaced with PBS.

Quantitative immunohistochemical analysis of HLA-DR expression was performed by image analysis using an Olympus BX41 trinocular microscope coupled with a CCD Samsung™ color camera (SDC-313/243) and PCTV software from the Pinnacle Media Center™. The images were analyzed using the Image J software (version 1.41, National Institutes of Health, USA)[19]. Cells were considered to be positive for the marker if a brown immunoprecipitate was present in the cytoplasm and/or on the cytoplasmic membrane.

The cell counter method was used to count labeled cells directly from the monitor of the computer; marks of different colors were placed on the positive and negative cells with a click of the mouse. After the count, all data were exported to Excel 2007® spreadsheets (Microsoft Corporation, USA), which provide the sum of the results of the count on one slide. At least 600 cells per biopsy were counted and the percentage of cells showing immunohistochemical expression of HLA-DR was calculated.

The statistical analysis was performed using the Fisher´s Exact test and ANOVA (for differences between percentages of stained cells). Significant differences were considered when $p<0.05$.

Results

Biopsy samples were obtained from 31 eyes, from 31 patients (22 male), with ages ranging from 50 to 76 years (median=66 years). Twenty five eyes were under prostaglandin analogue treatment (Group 1) and six were under the use of other pharmacological agents (Group 2) during the 30 days prior to the surgical procedure. The number of antiglaucoma eye drop preparations in use ranged from 1 to 4 (median 3) per eye, and the most frequently used medication was 0.5% timolol maleate (27 eyes).

Fourteen eyes of Group 1 (56%) and three of Group 2 (50%) presented conjunctival expression of the inflammatory marker HLA-DR (Figure 1). The percentage of stained cells ranged from 15.49 to 48.09% (median: 27.61) in Group 1 and from 18.35 to 28% (median=20.71) in Group 2, and these differences were not statistically significant (p=0.33; IC 95%: 0.213 to 7.585).

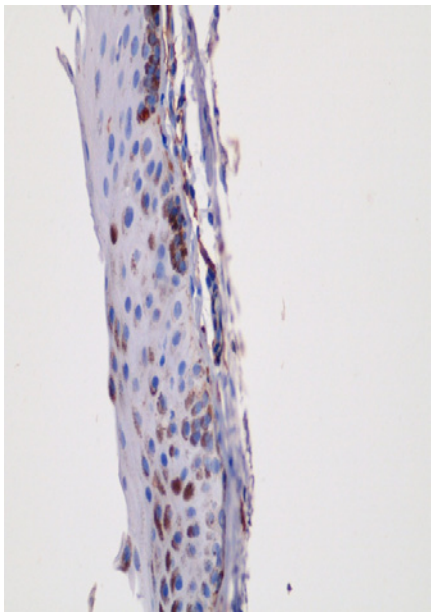

FIGURE 1 - Conjunctival biopsy showing positive labeling for the anti-HLA-DR antibody (arrow). Magnification 400x.

Discussion

In the present study, the use of prostaglandin analogues was not a risk factor for conjunctival HLA-DR expression. In healthy subjects, conjunctival expression of Human Leucocitary Antigens (HLA) is low. But in patients under antiglaucomatous topical medication, HLA-DR and other inflammatory markers are detected even in the absence of clinical signs of inflammation, such as conjunctival hyperemia[6-10,12-15].

Although most of the similar studies found in the literature were performed with patients in use of prostaglandin analogues, it is currently accepted that BAK, a preservative widely used in ophthalmic preparations, play an important role in conjunctival inflammation[20].

Recently, it was described that patients presenting ocular side-effects due to topic prostaglandin analogues had a better tolerability when switching to a preservative-free prostaglandin solution (tafluprost)[17]. But although the percentage of patients presenting HLA-DR expression decreased, 87% of them were still expressing abnormal levels of HLA-DR after 12 weeks under preservative-free medication, which suggests that BAK is not the only factor involved in subclinical conjunctival inflammation.

In a previous publication, our group demonstrated a higher expression of conjunctival HLA-DR in eyes from patients in use of topic prostaglandin analogues for thirty or more days (94% of all eyes), although patients remained without any clinical sign of conjunctival inflammation[12]. In the current study, although most of the subjects under chronic medication presented conjunctival HLA-DR expression, the prevalence was lower (54.8% including all eyes, 56% considering only the group under prostaglandin analogues medication). This frequency could be explained by the differences in methodology (impression cytology versus biopsy). Another potential explanation could be that subclinical inflammation is more frequent in early stages of the treatment. Rodrigues *et al*.[12] observed HLA-DR expression in conjunctival cells 94% of the patients followed-up, after one month of treatment with prostaglandin analogues.

We decided to select patients with indication of trabeculectomy, so a conjunctival biopsy could be ethically justified at the moment of the surgery. That way, more cells can be analyzed than when an imprint is obtained.

One of the limitations of this study is the reduced number of patients, especially in Group 2. For ethical reasons, the patients remained with the maximum tolerated treatment until the day of the surgery, when the specimens were collected. As prostaglandin analogues currently are the first choice drugs in antiglaucoma therapy[3], most of the patients were under this type of medication. Thus, the present study was performed before the preservative-free prostaglandin tafluprost was commercially available, so the effect of this drug on conjunctival HLA-DR expression could not be evaluated.

The frequency of subclinical inflammation in both

groups suggests that this condition may be attributed to both, prostaglandin analogues and the vehicle of ophthalmic solutions used in glaucoma treatment (BAK). Further studies analyzing the expression of inflammatory markers in asymptomatic patients under preservative-free antiglaucoma medication should be performed in order to investigate if these medications, although causing less ocular symptoms, are able or not to produce subconjunctival inflammation and also to increase the risk of trabeculectomy failure.

Conclusion

HLA-DR conjunctival expression in eyes treated with prostaglandin analogs was not significantly different of the expression in eyes treated with other topical agents, used for glaucoma treatment.

References

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol. 2006;90:262-7.
2. Heijl A, Leske MC, Bengtsson B, Hyman L, Hussein M. Reduction of intraocular pressure and glaucoma progression: results from the Early Manifest Glaucoma Trial. Arch Ophthalmol. 2002;120:1268-79.
3. De Natale R, Le Pen C, Berdeaux G. Efficiency of glaucoma drug regulation in 5 European countries: a 1995-2006 longitudinal prescription analysis. J Glaucoma. 20:234-9.
4. Stewart WC, Stewart JA, Jenkins JN, Jackson AL. Corneal punctate staining with latanoprost, bimatoprost, and travoprost in healthy subjects. J Glaucoma. 2003;12:475-9.
5. Stewart WC, Kolker AE, Stewart JA, Leech J, Jackson AL. Conjunctival hyperemia in healthy subjects after short-term dosing with latanoprost, bimatoprost, and travoprost. Am J Ophthalmol. 2003;135:314-20.
6. Hong S, Lee CS, Seo KY, Seong GJ, Hong YJ. Effects of topical antiglaucoma application on conjunctival impression cytology specimens. Am J Ophthalmol. 2006;142:185-6.
7. Cvenkel B, Ihan A. Ocular surface changes induced by topical antiglaucoma monotherapy. Ophthalmologica. 2002;216:175-9.
8. Guglielminetti E, Barabino S, Monaco M, Mantero S, Rolando M. HLA-DR expression in conjunctival cells after latanoprost. J Ocul Pharmacol Ther. 2002;18:1-9.
9. Pisella PJ, Debbasch C, Hamard P, Creuzot-Garcher C, Rat P, Brignole F, Baudouin C. Conjunctival proinflammatory and proapoptotic effects of latanoprost and preserved and unpreserved timolol: an ex vivo and in vitro study. Invest Ophthalmol Vis Sci. 2004;45:1360-8.
10. Baudouin C, Liang H, Hamard P, Riancho L, Creuzot-Garcher C, Warnet JM, Brignole-Baudouin F. The ocular surface of glaucoma patients treated over the long term expresses inflammatory markers related to both T-helper 1 and T-helper 2 pathways. Ophthalmology. 2008;115:109-15.
11. Broadway DC, Grierson I, O'Brien C, Hitchings RA. Adverse effects of topical antiglaucoma medication. II. The outcome of filtration surgery. Arch Ophthalmol. 1994;112:1446-54.
12. Rodrigues Mde L, Felipe Crosta DP, Soares CP, Deghaide NHS, Duarte R, Sakamoto FS, Furtado JMF, Paula JS, Donadi EA, Soares EG. Immunohistochemical expression of HLA-DR in the conjunctiva of patients under topical prostaglandin analogs treatment. J Glaucoma. 2009;18:197-200.
13. Nuzzi R, Vercelli A, Finazzo C, Cracco C. Conjunctiva and subconjunctival tissue in primary open-angle glaucoma after long-term topical treatment: an immunohistochemical and ultrastructural study. Graefes Arch Clin Exp Ophthalmol. 1995;233:154-62.
14. Ihan A, Cvenkel B. Conjunctival epithelium expression of HLA-DR in glaucoma patients and its influence on the outcome of filtration surgery. Br J Ophthalmol. 2000;84:648-50.
15. Baudouin C, Hamard P, Liang H, Creuzot-Garcher C, Bensoussan L, Brignole F. Conjunctival epithelial cell expression of interleukins and inflammatory markers in glaucoma patients treated over the long term. Ophthalmology. 2004;111:2186-92.
16. Guenoun JM, Baudouin C, Rat P, Pauly A, Warnet JM, Brignole-Baudouin F. In vitro study of inflammatory potential and toxicity profile of latanoprost, travoprost, and bimatoprost in conjunctiva-derived epithelial cells. Invest Ophthalmol Vis Sci. 2005;46:2444-50.
17. Uusitalo H, Chen E, Pfeiffer N, Brignole-Baudouin F, Kaarniranta K, Leino M, Puska P, Palmgren E, Hamacher T, Hofmann G, Petzold G, Richter U, Riedel T, Winter M, Ropo A.Switching from a preserved to a preservative-free prostaglandin preparation in topical glaucoma medication. Acta Ophthalmol. 2010;88:329-36.
18. Tsironi S, Ioachim E, Machera M, Aspiotis M, Agnantis N, Psillas K. Immunohistochemical HLA-DR antigen expression with lymphocyte subsets and proliferative activity in pterygium. In Vivo. 2002;16:299-306.
19. Girish V, Vijayalakshmi A. Affordable image analysis using NIH Image/ImageJ. Indian J Cancer. 2004;41:47.
20. Baudouin C, Labbe A, Liang H, Pauly A, Brignole-Baudouin F. Preservatives in eyedrops: the good, the bad and the ugly. Prog Retin Eye Res. 29:312-34.

Acknowledgments

The authors express their gratitude to Régia Carolina Lira MSc and Ana Maria Rocha BSc for technical support.

Correspondence:
Profa. Dra. Maria de Lourdes Veronese Rodrigues
Hospital das Clinicas, Oftalmologia
Campus USP
14048-900 Ribeirão Preto-SP Brasil
mdlvrodr@fmrp.usp.br

Received: May 14, 2012
Review: July 16, 2012
Accepted: August 17, 2012
Conflict of interest: none
Financial source: none

[1]Research performed at Glaucoma Clinic, University Hospital, Faculty of Medicine of Ribeirao Preto-University of Sao Paulo (FMRP-USP), Brazil. Part of PhD thesis, Postgraduate Program in Ophthalmology, Otorhinolaringology and Head and Neck Surgery, FMRP-USP. Tutor: Profa. Dra. Maria de Lourdes Veronese Rodrigues.