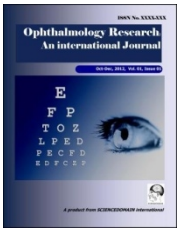

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Postoperative Use of Cyclosporine, Prednisolone or a Cyclosporine/Prednisolone Combination to Treat Inflammation of the Conjunctiva Following Trabeculectomy in Rabbits

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Authors' contributions

This work was carried out in collaboration between all authors. Author HHR designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors NVLF and FMF managed the literature searches and analyses of the study and authors HHR and PR managed the experimental process. Authors PAAM and SC wrote final revision. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Objectives: The objective of this investigation was to compare different topical treatment protocols for controlling inflammation in rabbits during the postoperative period following trabeculectomy.
Methods: Thirty rabbits had their right eyes treated with a daily eye drop of 0.03% bimatoprost for 30 days. All rabbits then underwent a trabeculectomy procedure and were divided into three groups (G1, G2 and G3). Rabbits from G1 received prednisolone acetate 1.0% four times a day, G2 received cyclosporine 0.05% twice a day and G3

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received both prednisolone acetate 1.0% four times a day and cyclosporine 0.05% twice a day. Seven days after the surgery all rabbits were euthanized and enucleated. Histomorphometry was performed in HE-stained sections to evaluate epithelial thickness, blood vessel diameter and vascular area. Immunohistochemical staining using monoclonal antibodies against smooth muscle actin, factor VIII and IL-6 was performed to assessfibrosis, neovascularization and inflammatory cell infiltration, respectively.
Results: In group G3 there was a reduction in the thickness and diameter of the conjunctival epithelium and the diameter and number of blood vessels, and less inflammatory infiltrate and fibrosis.
Conclusion: This study suggests that a combination of prednisolone and cyclosporine is more effective than either of these drugs used alone to control postoperative conjunctival inflammation following trabeculectomy in rabbits.

Keywords: Conjunctiva; glaucoma; drug therapy; histology; histomorphometry; immunohistochemistry.

1. INTRODUCTION

Glaucoma is a multifactorial progressive optical neuropathy characterized by structural changes in the optic disc. It is frequently accompanied by changes in the field of vision and requires treatment over a long period with topical hypotensive drugs [1,2].Various classes of drugs are commercially available to treat this disease, including cholinergics, beta blockers, alpha-adrenergic agonists, carbonic anhydrase inhibitors and, more recently, prostaglandin analogs [3].

In patients treated with topical drugs, the conjunctiva acts as a semipermeable membrane, allowing these active ingredients to be absorbed and exert their action within the eye. However, with the chronic use of topical treatment the conjunctiva responds with scar tissue formation, keratinization and neovascularization, which can change the shape of the membrane and the way it operates [4]. The intensity of these side effects depends on the duration of treatment as well as the concentration and amount of drugs used [5].

Russ et al.[6] characterized the inflammatory conjunctival changes observed in rabbits after 30 days of a topical glaucoma treatment regimen using timolol maleate or prostaglandin analogues [6]. In the group treated with timolol, there was a significant increase in conjunctival epithelial thickness and a marked increase in subepithelial collagen density, a finding that is consistent with the literature [4,5]. The authors considered this finding as secondary to epithelial cell edema or the presence of an inflammatory infiltrate and epithelial cell injury. The number and diameter of blood vessels were also evaluated. An increase in the number of small-diameter blood vessels, probably due to neovascularization, was observed by the same investigators [6].

There is solid evidence that these changes on the surface of the conjunctiva can increase the risk of surgical failure in glaucoma patients who undergo filtering surgery, such as trabeculectomy [5,7,8].

Broadway et al. [5] identified subclinical conjunctival inflammation due to prior topical therapy as a risk factor for unsuccessful trabeculectomy. The reduction in the success rate for glaucoma filtering procedures can probably be attributed to the activation of fibroblasts in the conjunctiva, Tenon’s capsule and episclera, leading to scar tissue formation around the

scleral flap [9,10,11].In the present study, a preoperative protocol consisting of topical bimatoprost (0.03%) was used to simulate conditions that adversely affect filtering surgery as described by this group in a previous study [6].Trabeculectomy is the most frequently used procedure for glaucoma [12,13] and is unique in that its success is dependent on interruption of the wound-healing response so that patency of the new filtration pathway is maintained. A completely healed trabeculectomy is a failed trabeculectomy. In the postoperative period following the surgery, various tissue repair processes take place, as in any surgical procedure. The initial processes involved in healing are inflammation andclotting, leading to a cascade of biological events including the release of cellular, hormonal and growth factors. These ultimately lead to tissue repair and scar tissue formation and are modulated by inflammatory mediators (histamine, serotonin, prostaglandin, leukotrienes and other kinines) that inducecells such as neutrophils, monocytes, macrophages and fibroblasts to migrate to the healing area. Clotting factor activation leads to conversion of fibrinogento fibrin. White blood cell response is followed by fibroblast and endothelial cell migration. Fibroblasts produce collagen, elastin and mucopolysaccharides. Collagen cross-linking and myfibroblast transformation lead to the formation of supercoils, which is also accompanied by dehydration, leading to dense scar tissue formation. While this process is expected in other surgical procedures, in glaucoma filtering surgery healing is not desirable because the fistula should be kept patent to drain aqueous humor and, consequently, reduce intraocular pressure [14].The wound-healing pathway can be divided into four main phases: coagulative, inflammatory, proliferative and post-proliferative remodeling. Steroids, for example, interrupt the inflammatory phase whereas 5-fluorouracil interrupts the proliferative phase. The avoidance of unnecessary trauma through gentle handling of tissue during surgerycan also influence the degree of inflammation and scar tissue formation. Although the gentle handling of tissue, control of intraoperative bleeding and use of postoperative anti-inflammatory agents lead to reduced fibroblastic activity, they are usually insufficient to prevent long-term scarring [14].

Adicks et al. [9] were the first to report that there is normally an area of collagenous connective tissue beneath the epithelium of a failed filtering bleb. This tissue is denser and thicker than in the rest of the entire bleb wall. Ultrastructurally this zone contains large amounts of collagen in which there are fibroblasts and thinner blood vessels. Functioning blebs have looser subepithelial connective tissue. Epithelial thickness and sub conjunctival vascularization, particularly small vessels, were parameters proposed by Francis et al. to evaluate filtering bleb biopsies[15]. Epithelial thickness was reduced in blebs with antifibrotics than in those without. The samewas observed with vascularity, which was reduced compared with acontrol bleb without any antimetabolite.

To avoid proliferation of fibroblasts and subconjunctival fibrosis, which cause the fistula to close in filtering surgery, antimetabolites are used to reduce the rate of cell mitosis [16,17] Mitomycin C (MMC) and 5-fluorouracil (5-FU) are the most commonly used drugs [15,18].

The use of these drugs increases the chances of continued patency of the fistula but also increases the likelihood of complications related to hypotonia and endophthalmitis [12,19,20].

Cyclosporine is an immunomodulatory drug that also has antimetabolic properties. It can control in vitro cell proliferation and has been studied in vivo in the postoperative period following trabeculectomy for some years [21,22].

Corticosteroids act by suppressing synthesis of inflammatory mediators and, consequently, reducing the number of leukocytes and fibroblasts at the inflammation site.They also affect vascular permeability [14]. The effect of these drugs in trabeculectomy has not yet been fully elucidated, but studies have shown that their topical use preoperatively and postoperatively leads to a greater reduction in intraocular pressure (IOP) and increases surgical success rates [14,18,23].

The purpose of this study is to compare three different post-trabeculectomy protocols based on cyclosporine and a corticosteroid (prednisolone acetate 1%, cyclosporine 0.05% and a combination of prednisolone and cyclosporine at these concentrations) by evaluating the subsequent inflammatory reaction.

2. MATERIALS AND METHODS

The experimental procedures followed the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research, and the study was approved by the Commissions for Animal Welfare at São Paulo University and Hospital Evangélico, Curitiba-PR, (CAPPESQ no. 161/05).

Thirty female New Zealand rabbits with similar ages (90 days) and weights (1.7kg) were treated with bimatoprost (0.03%) eye drop for thirty days. Only clinically healthy animals free of eye disease were included. All animals had been previously treated with an endectocide (Ivermectin, Merial Brasil, Campinas, São Paulo, Brazil) and received a ration supplemented with vitamins (ADE, Pfizer Saúde Animal, São Paulo, Brazil) in accordance with Hospital Evangélico´s vivarium rules.

One drop of the medication was instilled in the right cornea of each animal, and the left eye was used as a control. The eye drop used contain edbenzalkonium chloride (0.005%) as a preservative. On the thirtieth day trabeculectomy was performed in the right eye of all the animals without any antimetabolite by the same surgeon.

Trabeculectomy was performed as follows: after insertion of corneal traction suture, an opening was made in the conjunctiva and Tenon's capsule with Westcott scissors. The base of the flap was located at the corneal limbus. After the conjunctiva was dissected, the scleral bed was exposed and bipolar diathermy was used to produce hemostasis. A rectangular limbus-based scleral flap measuring about 4x3 mm and approximately half as thick as the sclera was made using a surgical blade number 11 (Feather Safety Razor Co. Ltda, Osaka, Japan) and a scleral crescent blade (Asico LLC, Westmont, USA). A block of limbal tissue including a portion of the trabecular meshwork and edge of the cornea was resected with a 0.75 scleral punch (Katena Products Inc. New Jersey, USA).After the block was removed, peripheral iridectomy was performed using Vannas scissors. Subsequently, the scleral flap was sutured with nonabsorbable thread (10.0 nylon) with two to four stitches, and the conjunctiva and Tenon's capsule were sutured with absorbable thread in a continuous pattern (11). All surgical procedures were carefully performed as the surgeon's experience of the surgical technique, the individual's systemic basal inflammatory reaction and excessive intraoperative bleeding, excessive cauterization and incorrect flap thickness are factors that can interfere with and damage the results of a trabeculectomy.

The rabbits were then divided randomly into three groups of ten animals (G1, G2 and G3).

The three postoperative protocols were as follows: prednisolone acetate 1% four times a day (G 1); cyclosporine 0.05% twice a day (G 2); and a combination of prednisolone acetate 1% four times a day and cyclosporine 0.05% twice a day (G 3). All the groups were given antibiotic therapy with gatifloxacin (0.03%) eye drops four times a day. Prednisolone acetate 1% and gatifloxacin are standard protocols after trabeculectomy at Hospital Evangélico. The acetate form of prednisolone penetrates the cornea far better than the phosphate form because of its biphasic solubility [14] and is therefore superior for control of intraocular inflammation [14].

After one week the animals were euthanized. The eyeballs were immediately fixed in 10% formaldehyde for 24 hours. After fixation, the eyes were sectioned, and the sections were dehydrated in a graded alcohol series (up to absolute alcohol), cleared with xylol and embedded in paraffin [24]. Sections cut from the paraffin block with a microtome were then stained with HE (hematoxylin-eosin) and assessed by immunohistochemistry using monoclonal antibodies against the following markers: factor VIII, smooth muscle actin and IL-6.

Histomorphometric analysis was performed with Image Pro-Plus version 4.5 software (Media Cybernetics®, Silver Spring, MD). Digital images were captured at 400x magnification and stored using the same program. Two points corresponding to the bulbar conjunctiva closest to the corneal limbus (Fig. 1) were selected on each slide. After the images had been captured, two segments of the conjunctival epithelium 200 µm long and 5 µm thick were selected at random from each photograph. By measuring linear segments of the same size, the diameter of the blood vessels and the thickness of the conjunctival epithelium could be determined with the aid of virtual tools included in the program.

Immunohistochemical analysis was carried out with images captured at 400x magnification using the same software. A virtual grid 150 X 200 µm was applied to each image and included the epithelium and lamina propria. The three subfields closest to the epithelium were then examined to allow a more specific analysis of the subepithelial region of the conjunctiva rather than the conjunctival lamina propria on each slide. This analysis was carried out twice for each slide for all parameters. The anti-actin antibody revealed the degree of inflammatory fibrosis (fibroblasts marked by the antibody). This parameter was evaluated using qualitative categorical variables, and numbers were assigned according to the staining intensity of the marker (1: minimum; 2: mild; 3: moderate; and 4: strong) in the histological section for each 400x field [25].

Factor VIII is a marker of vascular endothelial cells and therefore of blood vessel walls in the conjunctival tissue. The degree of neovascularization was determined by counting the number of marked blood vessels in each 400x field. Two counts were taken for each slide. The degree of inflammatory infiltration was determined by counting the areas stained with anti-interleukin-6 antibody in the histological section. The person responsible for reading the slides was blinded to the identities of the groups. The parameters were measured twice by the same investigator, and the coefficients of variability between the two sets of readings were low.

The categorical variables were analyzed with Fisher's exact test and presented as medians. One-way ANOVA with a 5% significance level was used to compare the continuous variables and was followed by Fisher's post-test (for comparisons between up to three groups) or the Tukey-Kramer test (for comparisons between more than three groups).

Statistical significance was defined as $p<0.05$. Continual variables were presented as means ± standard deviations.

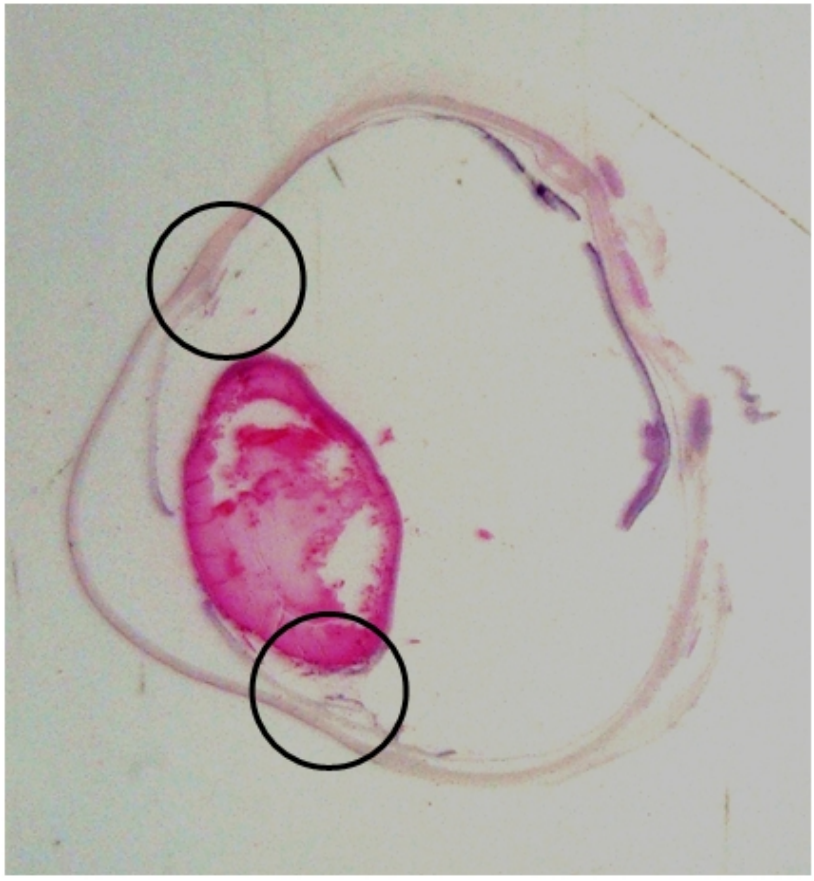


Fig. 1. Photograph of an HE-stained histological slide showing the two areas of bulbar conjunctiva close to the ventral and dorsal limbus (circled) that were assessed microscopically

3. RESULTS

In the histomorphometric assessment, the group treated with prednisolone had a thicker conjunctival epithelium (*P*=0.03); the means for the prednisolone, cyclosporine and prednisolone+cyclosporine groups were 29.3 ± 10.27 µm; 25.1 ± 5.16 µm; and 24.4 ± 3.81, respectively. The diameters of the blood vessels were significantly larger in the animals treated with prednisolone (30.3 ± 11.28 µm) or cyclosporine (27.7 ± 10.90 µm) (*P*=0.0011 and *P*<0.0001, respectively) than in the group treated with a combination of the two drugs (19.8 ± 6.9 µm) (Fig. 2; Table 1).

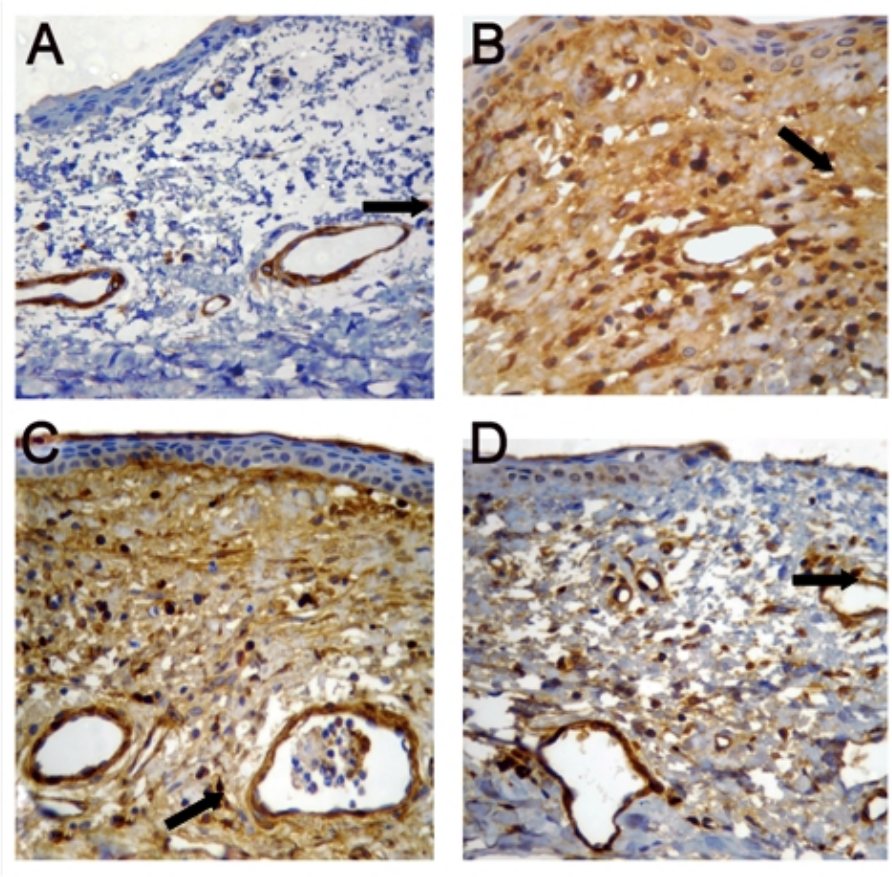
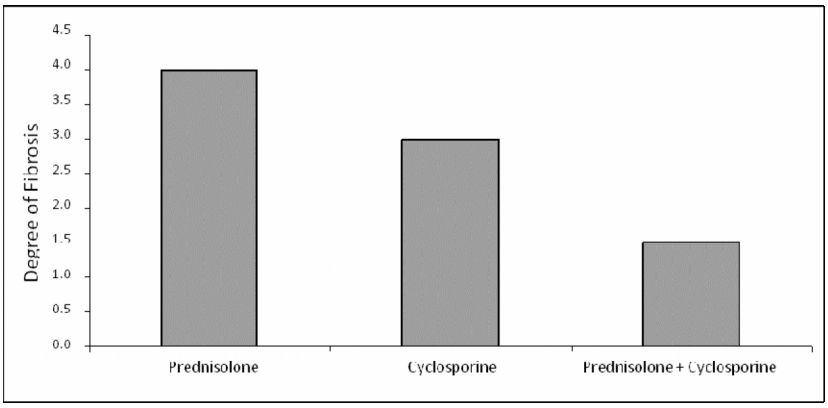


Fig. 2. Photomicrographs of conjunctiva (400 x magnification) stained with anti-actin antibody. Subepithelial fibroblasts can be seen for the control group (A) and groups G1 (B), G2 (C) and G3 (D). Note the smaller number of fibroblasts in the eye treated with cyclosporine + prednisolone (D) than in the eye treated with prednisolone alone (B)

Fibrosis was also less intense in the group that received the combination treatment (median 1.5) than in the groups treated with prednisolone (median 4.0) or cyclosporine (median 3.0) on their own (P<0.001) (Graph 1).



Graph 1. Comparison of the degree of conjunctival fibrosis in the three treatment groups: prednisolone 1.0% (G1), cyclosporine 0.05% (G2) and prednisolone 1.0% with cyclosporine 0.05% (G3). Note the less intense fibrosis in the group that received the combination treatment. Immunohistochemical assessment using anti-actin antibody and discrete numeric values (1: minimal; 2: mild; 3: moderate; and 4: intense)

Factor VIII revealed less vascularization in response to the inflammatory process in G3 (8.07) than in G1 (17.50) or G2 (11.86) (P<0.001) (Table 1 and Fig. 3).

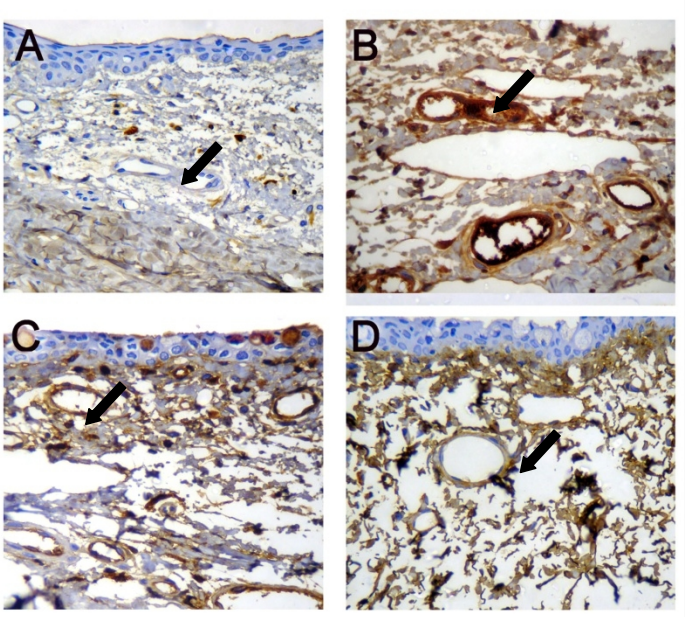


Fig. 3. Photomicrographs of conjunctivae (400 x magnifications) stained with anti-factor VIII antibody. The endothelial cells in the blood vessels formed in response to the inflammatory process can be seen for the control group (A) and groups G1 (B), G2 (C) and G3 (D). Note the greater number of vessels in the eye treated with prednisolone alone (B) than in the eye treated with prednisolone+cyclosporine (D)

Table 1. Comparison of the parameters measured for the three treatment groups: prednisolone 1% (G1), cyclosporine 0.05% (G2) and prednisolone 1.0% with cyclosporine 0.05% (G3)

	Thickness of the conjunctival epithelium	**P**	**Diameter of the blood vessels**	**P**	**Vascularization (no. of vessels)**	**P**	**Anti-interleukin 6**	**P**
Prednisolone (G1)	29.3 ± 10.27 µm	P<0.03	30.3 ± 11.28 µm	*P*<0.0011	17.50	*P*<0.001	27.37	*P*=0.0005
Cyclosporine (G2)	25.1 ± 5.16 µm		27.7 ± 10.90 µm	*P*<0.0001	11.86	*P*<0.001	13.20	*P*<0.001
Prednisolone + Cyclosporine (G3)	24.4 ± 3.81		19.8 ± 6.9 µm		8.07		6.60	

In the immunohistochemical assessment using anti-interleukin 6, less inflammatory infiltrate was observed in G3 (6.60) than in G1 (27.37) (P=0.0005) or G2 (13.20) (P<0.001) (Table 1 and Fig. 4).

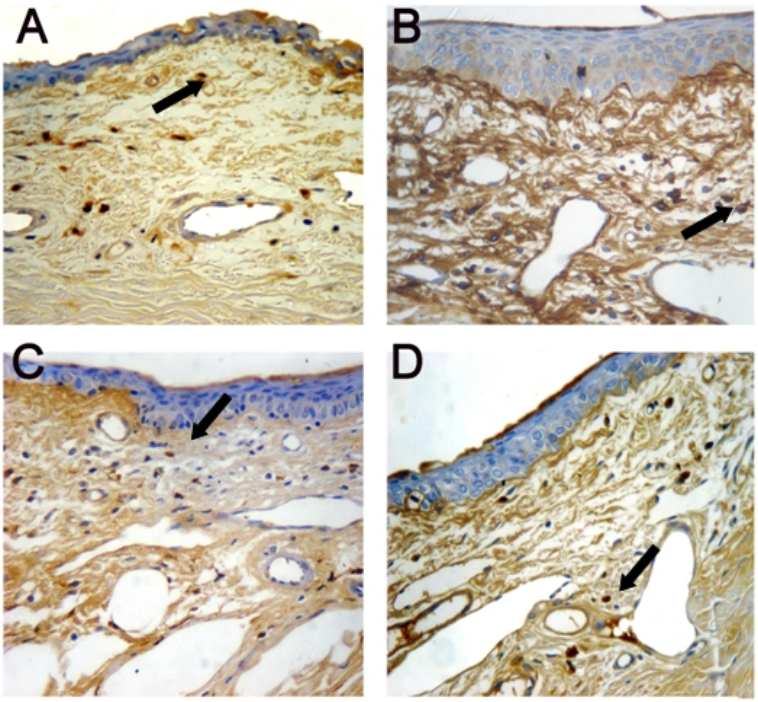


Fig. 4. Photomicrographs of conjunctivae (400 x magnifications) stained with anti-interleukin-6 antibody. Inflammatory subepithelial cells can be seen for the control group (A) and groups G1 (B), G2 (C) and G3 (D). Note the smaller number of inflammatory cells in the eye treated with cyclosporine+prednisolone (D) than in the eye treated with prednisolonealone (B)

4. DISCUSSION

Trabeculectomy has been used to control glaucoma for over forty years. It has a relatively high success rate and is still the most commonly used incisional technique to treat the disease5,20.

Many clinical and experimental studies have reported strong evidence that chronic use of topical glaucoma drugs can induce changes in the conjunctival surface, causing discomfort during instillation, conjunctival inflammation, tear film instability, subconjunctival fibrosis, apoptosis of conjunctival epithelial cells and changes in the corneal surface [6,26,27,28]. Some researchers suggest that these changes result in a worse prognosis for trabeculectomy in patients who had previously been receiving topical glaucoma therapy [4,5,11,29,30]. In clinical, laboratory and experimental studies, many of these conjunctival changes have been associated not only with glaucoma drugs themselves but also with benzalkonium chloride (BAK), the most commonly used preservative in topical ophthalmic preparations [7,21].

In the present study, prior treatment with bimatoprost, in which BAK is used as the preservative, resulted in side effects in the conjunctiva. These have already been reported by other authors and include changes in epithelial thickness, conjunctival inflammatory cell infiltration, vascularization and fibrosis. In another study by the same group of authors these changes were evidenced in the bimatoprost group after 30 days of a daily drop treatment [6,31].

The most frequent cause of failure in trabeculectomy is the formation of episcleral fibro vascular tissue around the surgical flap [32]. We observed that the synergic action of cyclosporine and prednisolone induced less fibrosis than each drug inisolation, thereby presumably preventing this tissue adhering to the flap and ensuring better surgical patency and controlled IOP as described by other authors [16,23].

The worst results for epithelial thickness were observed with prednisolone, which resulted in more conjunctival edema and inflammatory infiltration. We can assume that cyclosporine alone or associated with a corticosteroid induces a less intense inflammatory reaction. Broadway et al. [33] used topical corticosteroids post-trabeculectomy and concluded that they were ineffective in suppressing subclinical conjunctival inflammation caused by the glaucoma therapy these patients had already been receiving, which corroborates the findings ofour study.

Recent studies have shown that cyclosporine induces a reduction in fibroblast proliferation in the conjunctival mucosa in rabbits [34,35] Cristofanilli et al. [22] showed that cyclosporine was able to control cell proliferation by inducing in vitro apoptosis, suggesting that it could be of benefit in post-trabeculectomy anti fibrosis therapy and leads to fewer complications. This inhibition of the proliferation of fibroblasts, endothelial cells and inflammatory cells such as mast cells explains the reduced fibrosis, vascularization and inflammatory infiltration observed in the group that received cyclosporine on its own and in the group that received the combination of cyclosporine and prednisolone.

The postoperative use of corticosteroids after eye surgery has been routine for several decades. However, the beneficial effects of using corticosteroids only started to be studied in the ’80s. Corticosteroids inhibit the inflammatory response by reducing vascular permeability and the amount of inflammatory mediators released. The decrease in vascular permeability in turn leads to a reduction in the concentration of lymphocytes, monocytes and basophils and a fall in macrophage activity at the inflammation site. This cascade of events inhibits mitotic activity, the release of growth factors and the production of fibrin in the lesion, culminating in a reduction in fibroblast activity and, therefore, inflammatory healing [14].

In the present study, the results for the vascular phase of inflammation (number and diameter of blood vessels as well as neovascularization) were worse for each drug used on its own than for a combination of the two [31,36].

The ineffectiveness of post-inflammatory treatment control with isolated corticosteroids and the treatment side effects caused by the use of antimetabolites in glaucoma surgery, such as infection (blebitis and endophthalmitis) and leakage from the bleb, leading to hypotonia and loss of visual acuity [17,20] justify research into new pre-, intra- and postoperative protocols. The “state of art” in inflammatory control in glaucoma surgery has yet to be achieved.

Our study has shown that the inflammatory parameters assessed here by histomorphometry (epithelial thickness and diameter of the blood vessels) were less intense for the group

treated with cyclosporine alone than for the group treated with prednisolone alone. The same was true of the parameters assessed by immunohistochemistry (degree of inflammatory infiltration and fibrosis and the number of vessels formed in response to the inflammatory process). All the parameters assessed had lower mean values for treatment with the drugs on their own than for treatment with the cyclosporine+prednisolone combination, suggesting that these drugs act synergistically to reduce the inflammatory response induced by topical therapy in the postoperative period following trabeculectomy.

Topical corticosteroid therapy with fluorometholone 1% or prednisolone 1.0% prior to trabeculectomy is quite effective for this purpose but, like other corticosteroids, can lead to cataracts and infections, particularly keratitis [13,34]. As the antiproliferative properties of corticosteroids take time to have an effect, some authors recommend that they be administered preoperatively so that they can be more effective in the postoperative period 37 Further studies into the effectiveness of corticosteroids and other drugs such as cyclosporine or tacrolimus in controlling preoperative inflammation of the conjunctiva caused by prior drug therapy are required.

5. CONCLUSION

The synergistic action of cyclosporine and prednisolone appears to be beneficial and to promote a greater reduction in conjunctival inflammation, fibrosis and neovascularization.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee (Commissions for Animal Welfare at São Paulo University and Hospital Evangélico, Curitiba-PR, CAPPESQ no. 161/05) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham EJ, Johnson CA, et al. The Ocular Hypertension Treatment Study: Baseline factors that predict the onset of primary open-angle glaucoma. Archives of Ophthalmology, 2002;120(6):714-20. Comment in: Archives of Ophthalmology. 2004;122(7):1088-9.

2. Weinreb RN; Medeiros FA. Como definir o glaucoma? In: Susanna Junior R, Weinreb RN. Glaucoma: Perguntas e Respostas. 1ª ed. Rio de Janeiro: Cultura Médica. 2005;1-7.
3. Ritch R. Shields B, Krupin T. Pharmacology. In: Ritch R, Shields MB, Krupin T. The glaucomas. 2ª ed. St. Louis: Mosby. 1996;3:1375-1489.
4. Brandt JD, Wittpenn JR, Katz LJ, Steinmann WN, Spaeth GL. Conjunctival impression cytology in patients with glaucoma using long-term topical medication. American Journal of Ophthalmology. 1991;112(3):297-301.
5. Broadway DC, Grierson I, O'Brien C, Hitchings R. A. Adverse effects of topical antiglaucoma medication. I. The conjunctival cell profile. Archives ofOphthalmology. 1994;112(11):1437-45.
6. Russ HH, Costa VP, Montiani-Ferreira F, Valgas SR, Correa Neto MA, Strobel EL, Truppel JH. Conjunctival Changes Induced by Prostaglandin Analogues and Timolol Maleate: A Histomorphometric Study. Arquivos Brasileiros de Oftalmologia. 2007;70(6):910-16.
7. Baudouin C, Garcher C, Haouat N, Bron A, Gastaud P. Expression of inflammatory membrane markers by conjunctival cells in chronically treated patients with glaucoma. Ophthalmology. 1994;101(3):454-60.
8. MietzH, Niesen U, Krieglstein GK. The effect of preservatives and antiglaucomatous medication on the histopathology of the conjunctiva. Graefe's Archive for Clinical and Experimental Ophthalmology. 1994;232(9):561-65.
9. Addicks EM, Quigley HA, Green WR, Robin AL. Histologic characteristics of filtering blebs in glaucomatous eyes. Archives of Ophthalmology. 1983;101(5):795-8.
10. Young LT, Higginbotham EJ, Zou XL, Farber MD. Effects of topical glaucoma drugs on fistulized rabbit conjunctiva. Ophthalmology. 1990;97(11):1423-27.
11. Herreras JM, Pastor JC, Calonge M, Asensio VM. Ocular surface alteration after long-term treatment with antiglaucomatous drug. Ophthalmology. 1993;99(7):1082-8
12. Susanna Junior R, Medeiros FA. Trabeculectomia. In: Susanna Junior R.Cirurgia do Glaucoma. 2ª ed. São Paulo: Rocca, 2002;27-64.
13. Khaw PT, Chang L, Wong TT, Mead A, Daniels JT, Cordeiro MF. Modulation of wound healing after glaucoma surgery. Curr Op Ophthal. 2001;12(2):143-8.
14. Lama PJ, Fechtner RD. Antifibrotics and wound healing in glaucoma surgery. Survey of Ophthalmology. 2003;48(3):314-46.
15. Francis BA, Du LT, Najafi K, Murthy R, Kurumety U, Rap N, Minckler DS. Histopathologic features of conjunctival filtering blebs. Archives of Ophthalmology. 2005;123(2):166-170.
16. Vijava L, Mukhesh BN, Shanta B, Ramalingam S, Sathi Devi AV. Comparison of lowdose intraoperative mitomycin-C vs 5-fluorouracil in primary glaucoma surgery: a pilot study. Ophthalmic Surgery & Lasers. 2000;31(1):24-30.
17. Bindlish R, Condon GP, Schlosser JD, D' Antonio J, Lauer KB, Lehrer R. Efficacy and safety of mitomycin-C in primary trabeculectomy: five-year follow-up. Ophthalmology. 2002;109(7):1336-41.
18. Moura CR, Mello PAA. Tratamientoquirúrgicodel glaucoma. In.: Mello PAA. Glaucoma. 1ªed, Quito: Nocion. 1999;201-221.
19. Wu Dunn D, Cantor LB, Palanca-Capistrano AM, Hoop J, Alvi NP, Finlev C, Lakhani V, Burnstein A, Knotts SL. Long-term outcomes of intraoperative 5-fluorouracil versus intraoperative mitomycin C in primary trabeculectomy surgery. American Journal of Ophthalmology. 2002;134(4):521-8.
20. Fontana H, Nouri-Mahdavi K, Lumba J, Ralli M, Caprioli J. Trabeculectomy with mitomycin C: outcomes and risk factors for failure in phakic open-angle glaucoma. Ophthalmology. 2006;113(6):930-6.

21. Turacli ME, Gunduz K, Aktan G, Tamer C. A comparatve clinical trial of mitomycin C and cyclosporine A in trabeculectomy. European Journal of Ophthalmology. 1996;6(4):398-401.
22. Cristofanilli M, Pescosolido N, Risuleo G, Scarsella G. A murine cell culture model for post-trabeculectomy antifibrotic treatment: induction of apoptosis by Cyclosporin. Acta Ophthalmologica Scandinavica. 2001;79(3):309-12.
23. Kahook MY, Camejo L, Noecker RJ. Trabeculectomy with intraoperative retrobulbar triamcinolone acetonide. Clin Ophthalmol. 2009;3:29-31.
24. BachaJr WJ, Wood LM. Principios generales de histologia. In.: BachaJr WJ, Wood LM. Atlas Color de Histologia Veterinaria. 1ªed. Buenos Aires: Intermédica. 1991;1-8.
25. Faria NVL, Russ HH, Rose P, Noronha L, Mello PA, Montiani-Ferreira F, Cronemberger S. Conjunctival changes and inflammatory aspects in rabbits' conjunctivas induced by fixed combinations of prostaglandin analogues and timolol maleate. Journalof Ophthalmic Inflammation and Infection. 2013;3-22.
26. Sherwood MB, Grierson I, Millar L, Hitchings RA. Long-term morphologic effects of antiglaucoma drugs on the conjunctiva and Tenon's capsule in glaucomatous patients. Ophthalmology. 1989;96(3):327-35.
27. De Saint Jean M, Brignole F, Bringuier AF, Bauchet A, Feldmann G, Baudouin C. Effects of benzalkonium chloride on growth and survival of Chang conjunctival cells. Investigative Ophthalmology Visual Science. 1999;40(3):619-30.
28. Bensoussan L, Blondin C, Baudouin C, Hamard P, Sabeh Afaki G, Creuzot-Garcher C, et al. Flow cytometric analysis of HLA-DR, IL-6 and IL-8 expression by conjunctival epithelial cells from patients with prolonged topical antiglaucoma treatments. Journal Françaisd' Ophtalmologie. 2003;26(8):782-89.
29. Lavin MJ, Wormald RP, Migdal CS, Hitchings RA. The influence of prior therapy on the success of trabeculectomy. Archives of Ophthalmology. 1990;108(11):1543-48.
30. Pfeiffer N, Griesrson I, Goldsmith H, Hochgesand D, Winkgen-Böhres A, Appleton P. Histological effects in the iris after 3 months of latanoprost therapy: the Mainz 1 study. Archives of Ophthalmology. 2001;119 (2):191-96.
31. Baudouin C, Pisella PJ, Fillacier K, Goldschild M, Becauet F, De Saint Jean M, Béchetoille A. Ocular surface inflammatory changes induced by topical antiglaucoma drugs. Ophtalmology. 2000;106(3):556-63.
32. Mietz H, Jacobi PC, Welsandt G, Krieglstein GK. Trabeculectomies in fellow eyes have an increase risk of Tenon's capsule cysts. Ophthalmology. 2002;109(5):992-7.
33. Broadway DC, Grierson I, O'Brien C, Hitchings RA. Adverse effects of topical antiglaucoma medication. II. The outcome of filtration surgery. Archives ofOphthalmology. 1994;112(11):1446-54. Comment in: Archives of Ophthalmology. 1994;113(7):849-50.
34. Leonardi A, De Franchis G, Fregona IA, Violato D, Plebani M, Secchi AG. Effects of cyclosporin A on human conjunctival fibroblasts. Archives of Ophthalmology. 2001;119(10):1512-17.
35. Kunert KS, Tisdale AS, Gipson HK. Goblet cell number and epithelial proliferation in the conjunctiva of patients whit dry eye syndrome treated whit cyclosporine. Archives of Ophthalmology. 2002;120(3):330-7.
36. Broadway DC, Grierson I, Stürmer J, Hitchings RA. Reversal of topical antiglaucoma medication effects on the conjunctiva. Archives of Ophthalmology. 1996;(114):262-67.

37. Nguyen KD, Lee DA. Effects of steroids and nonsteroidal antiinflamatory agents on human ocular fibroblast. Investigative Ophthalmology & Visual Science. 1992;33:2693-2701.

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