

**POSTOPERATIVE USE OF CYCLOSPORINE, PREDNISOLONE OR A
CYCLOSPORINE/PREDNISOLONE COMBINATION TO TREAT
INFLAMMATION OF THE CONJUNCTIVA FOLLOWING
TRABECULECTOMY IN RABBITS**

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 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 were 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 evaluate fibrosis, neovascularization and inflammatory infiltrate, 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 efficient than any of these drugs used alone to control conjunctival post operative conjunctival inflammation following trabeculectomy in rabbits.

Keywords: conjunctiva, glaucoma, drug therapy, histology, histomorphometry, immunohistochemistry.

INTRODUCTION

Glaucoma is a multifactorial progressive optical neuropathy characterized by structural changes in the optic disc and frequently accompanied by changes in the field of vision that requires treatment over a long period with topical hypotensive drugs (Gordon et al., 2002; Weinreb and Medeiros, 2005). 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 (Ritch et al. 1996).

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 (Brandt et al., 1991). The intensity of these side effects depends on the length of treatment as well as the concentration and amount of drugs used (Broadway et al. 1994^{1,2}).

Russ et al (2007) characterized the inflammatory conjunctival changes observed in rabbits after 30 days of a topical glaucoma treatment regimen using timolol maleate or prostaglandin analogues (Russ et al, 2007). In the group treated with timolol, there was a significant increase in conjunctival epithelial thickness and a marked increase of subepithelial collagen density, a finding that is consistent with the literature (Broadway, 1994; Brandt, 2001). The authors considered this finding as secondary to epithelial cell edema, or to the presence of an inflammatory infiltrate and epithelial cell injury. Number and diameter of blood vessels also were evaluated. An increase in number of blood vessels of small diameter, probably due to neovascularization, was observed by the same investigators (Russ et al, 2007).

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 (Baudouin et al., 1994; Broadway et al., 1994²; Mietz et al., 1994).

Broadway et al. (1994¹) 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 (Addicks et al., 1983; Young et al., 1990; Herreras et al., 1993). 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 previous study (Russ et al, 2007). Trabeculectomy is the most frequently used procedure for glaucoma. (Susanna and Medeiros, 2002). Glaucoma surgery is unique in that its success is linked to interruption of the wound-healing response in order to maintain patency of the new filtration pathway. A completely healed trabeculectomy is a failed trabeculectomy. In the postoperative period following trabeculectomy, various tissue repair processes take place, as

in any surgical procedure. The initial processes involved in healing are inflammation and clotting, leading to a cascade of biological events including cellular, hormonal, and growth factor release. These ultimately lead to tissue repair and scar tissue formation. These are modulated by inflammatory mediators (histamine, serotonin, prostaglandin, leukotrienes and other kinins) that induce white blood cells such as neutrophils, monocytes, macrophages and fibroblasts to migrate to the healing area. Clotting factor activation leads to conversion of fibrinogen to fibrin. White blood cell response is followed by fibroblast and endothelial cell migration. Fibroblasts produce collagen, elastin, and mucopolysaccharides. Collagen cross-linking as well as myofibroblast transformation leads to collagen supercoil formation, 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 process are not desirable because the fistula should be kept patent to drain aqueous humor and, consequently, reduce intraocular pressure (Lama and Fechtner, 2003). 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 surgery also can influence the degree of inflammation and scar tissue formation. Whereas gentle handling of tissue, control of intraoperative bleeding, and use of postoperative anti-inflammatory agents leads to reduced fibroblastic activity, such maneuvers are usually insufficient to prevent scarring for the long term. (Lama, 2003).

Adicks et al (1983) first described beneath the epithelium of failure filtering bleb there is normally an area of collagenous connective tissue. This tissue was denser and thicker than normal throughout the entire bleb wall. Ultrastructurally this zone contained large amounts of collagen in which there were fibroblasts and thinner blood vessels. Functioning blebs had looser sub epithelial connective tissue. Epithelial thickness and subconjunctival vascularization, particularly small vessels, were parameters proposed for Francis et al (2005) to evaluate filtering blebs biopsies. Epithelial thickness measurement was lower in blebs with antifibrotics than without. The same was observed with vascularity, that was decreased when compared with a control bleb with no 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 (Vijava et al., 2000; Bindlish et al., 2002). Mitomycin C (MMC) and 5-fluorouracil (5-FU) are the most commonly used drugs (Moura and Mello, 1999; Francis et al., 2005).

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 (Susanna and Medeiros, 2002; Wu Dunn et al., 2002; Fontana et al., 2006).

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 (Turacli et al., 1996; Cristofanilli et al., 2001).

Corticosteroids act by suppressing synthesis of inflammatory mediators and, consequently, reducing the number of leukocytes and fibroblasts at the inflammation site besides their effects on vascular permeability (Lama and Fechtner, 2003). Until recently, the effect of these drugs in trabeculectomy has not been fully elucidated, but studies have shown that their topical use preoperatively and postoperatively enlarge the IOP reduction and increases surgical success rates (Moura and Mello, 1999; Lama and Fechtner, 2003; Kahook et al., 2009).

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.

MATERIAL 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 were previously treated with an ecto and endoparasiticide drug (also called endectocide) Ivermectin, Merial Brasil, Campinas, São Paulo, Brazil) and received vitamin supplementation (ADE, Pfizer Saúde Animal, São Paulo, Brazil), following Hospital Evangélico's vivarium rules.

One drop the medication was instilled in the right cornea of each animal, and the left eye was used as controls. The eye drop used contained benzalkonium chloride (0.005%) as a preservative. On the thirtieth day trabeculectomy was performed in the right eye without antimetabolites on all the animals by the same surgeon.

Trabeculectomy technique was performed as follows: after insertion of corneal traction suture, an opening in the conjunctiva and Tenon's capsule were performed using a Westcott scissor. The base of this flap was at the corneal limbus. After the conjunctiva is 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 scleral thickness was made using a surgical blade number 11 (Feather Safety Razor Co, Ltda, Osaka, Japan) and an scleral crescent blade (Asico LLC, Westmont, USA). A block of limbal tissue was resected including a portion of the trabecular meshwork and edge of the cornea with an scleral punch 0.75 (Katena Products Inc. New Jersey, USA). After block removal, peripheral iridectomy was performed using a Vannasscissor. Subsequently, the scleral flap was sutured with nonabsorbable thread (10.0 nylon) with two to four sutures, and the conjunctiva and Tenon's capsule are sutured with absorbable thread in a continuous pattern (11). All surgical procedures were carefully performed since surgeon's experience with surgical technique, individual's systemic basal inflammatory reaction, besides intraoperative excessive bleeding, excessive cauterization, misplaced flap thickness are factors that can interfere 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 were standard protocols at Evangelic University Hospital after trabeculectomy. The acetate form of prednisolone penetrates the cornea far better than the phosphate form as a result of its biphasic solubility (Lama et al, 2003). Thus, for control of intraocular inflammation, prednisolone acetate is superior to prednisolone phosphate (Lama et al, 2003).

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 (Bacha Jr. and Wood, 1991). 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 (Figure 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 in 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 (Faria et al, 2013).

Factor VIII is a marker of vascular endothelial cells and therefore of blood vessel walls in the conjunctival tissue. 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 infiltrate 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 $\pm$ standard deviations.

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 $29.3 \pm 10.27 \mu\text{m}$; $25.1 \pm 5.16 \mu\text{m}$; and 24.4 ± 3.81 , respectively. The diameters of the blood vessels were significantly larger in the animals treated with prednisolone ($30.3 \pm 11.28 \mu\text{m}$) or cyclosporine ($27.7 \pm 10.90 \mu\text{m}$) ($P=0.0011$ and $P<0.0001$, respectively) than in the group treated with a combination of the two drugs ($19.8 \pm 6.9 \mu\text{m}$) (Figure 2; Table 1). 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).

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 Figure 3).

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 Figure 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 disease (Broadway et al., 2004; Fontana et al., 2006).

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 (Sherwood et al., 1989; De Saint Jean et al., 1999; Bensoussan et al., 2003; Russ et al., 2007). Some researchers suggest that these changes result in a worse prognosis for trabeculectomy in patients who had previously been receiving topical glaucoma therapy (Lavin et al., 1990; Brandt et al., 1991; Herreras et al., 1993; Broadway et al., 1994; Pfeiffer et al., 2001). 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 (Baudouin et al., 1994; Turacli et al, 1996^{1,2}).

In the present study, previous 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 infiltrate, 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. (Baudouin et al. 1999; Russ et al., 2007).

The most frequent cause of failure in trabeculectomy is the formation of episcleral fibrovascular tissue around the surgical flap (Mietz et al., 2002). We observed the synergic action of cyclosporine and prednisolone induced fewer fibrosis than each isolated drug, which presumably can prevent this tissue to adhere to the flap, thus ensuring better surgical patency and controlled IOP as described by other authors (Vijava et al., 2000; Kahook et al, 2009).

Considering epithelial thickness, we observed worst results for the prednisolone which means more conjunctival edema and inflammatory infiltrate. We can assume that cyclosporine alone or associated with a corticosteroid induces a less intense inflammatory reaction. Broadway et al. (1994) used topical corticosteroids post-trabeculectomy and concluded that they were ineffective in suppressing subclinical caused by glaucoma therapy that these patients had already been receiving, which corroborates with our study.

Recent studies have shown that cyclosporine induces a reduction in fibroblast proliferation in the conjunctival mucosa in rabbits (Leonardi et al., 2001; Kunert et al., 2002). Cristofanilli et al. (2001) showed that cyclosporine was able to control cell proliferation by inducing in vitro apoptosis, suggesting that it could be of benefit in post-trabeculectomy antifibrosis therapy and leads to fewer complications. This inhibition of the proliferation of fibroblasts, endothelial cells and inflammatory cells such as mast cells explains the smaller degree of fibrosis, vascularization and inflammatory infiltrate observed both 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 also inhibit the inflammatory response

through reduction in the amount of inflammatory mediators, together with the decrease in vascular permeability caused by the drug, reduces the concentration of lymphocytes, monocytes and basophils and leads to 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 (Lama, 2003)

Concerning to vascular phase of inflammation (number and diameter of blood vessels as neovascularization), either drugs presented poorer results than combination of them. (Broadway, 1994; Kunert, 2000)

The ineffectiveness of post-inflammatory treatment control with isolated corticosteroids, and the threatening side effects caused by the use of antimetabolites in the glaucoma surgery such as infection (blebitis and endophthalmitis) and leakage from the bleb, leading to hypotony and loss of visual acuity (Bindlish et al., 2002; Fontana et al., 2006), justify the research of new operative protocols, even before, intra or post-surgery. A “state of art” inflammatory control in glaucoma surgery was not achieved yet.

Our study showed 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 infiltrate 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 (Broadway, 1996; Lama and Fechner, 2003). 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 (Nguyen and Lee, 1992).

Subsequent studies to investigate the effectiveness of corticosteroids and other drugs as cyclosporine or tacrolimus in controlling preoperative inflammation of the conjunctiva caused by prior drug therapy should be recommended.

CONCLUSION

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

REFERENCES

ADDICKS, E. M.; QUIGLEY, H.A.; GREEN, W.R.; ROBIN, A. L. Histologic characteristics of filtering blebs in glaucomatous eyes. **Archives of Ophthalmology**.v.101, n.5, p.795-8, 1983.

BACHA JR, W. J.; WOOD, L. M. Principios generales de histologia. In.: BACHA JR, W. J.; WOOD, L. M. **Atlas Color de Histologia Veterinaria**. 1a. ed. Buenos Aires: Intermédica. 1991, p.1-8.

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**.v.101, n.3, p.454-460, 1994.

BAUDOUIN, C.; PISELLA, P.J.; FILLACIER, K.; GOLDSCHILD, M.; BECAUET, F.; DE SAINT JEAN, M.; BÉCHETOILLE, A. Ocular surface inflammatory changes induced by topical antiglaucoma drugs. **Ophthalmology**. v.106, n.3, p.556-563, 1999.

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 Français d'Ophtalmologie**.v.26, n.8, p.782-789, 2003.

BINDLISH, R.; CONDON, G.P.; SCHLOSSER, J.D.; D'ANTONIO, J.; LAUER, K.B.; LEHRER, R. Efficacy and safety of mitomycin-C in primary trabeculectomy: five-year follow-up. **Ophthalmology**. v.109, n.7, p.1336-1341, 2002.

BRANDT, J. D.; WITTPENN, J. R.; KATZ, L. J.; STEINMANN, W.N.; SPAETH, G. L. Conjunctival impression cytology in patients with glaucoma using long-term topical medication. **American Journal of Ophthalmology**.v.112, n.3, p.297-301, 1991.

BROADWAY, D. C.; GRIERSON, I.; O'BRIEN, C.; HITCHINGS, R. A. Adverse effects of topical antiglaucoma medication. I. The conjunctival cell profile. **Archives of Ophthalmology**.v.112, n.11, p.1437-1445, 1994¹.

BROADWAY, D.C.; GRIERSON, I.; O'BRIEN, C.; HITCHINGS, R. A. Adverse effects of topical antiglaucoma medication. II. The outcome of filtration surgery. **Archives of Ophthalmology**.v.112, n.11, p. 1446-1454, 1994. Comment in: **Archives of Ophthalmology**. v.113, n.7, p.849-850, 1994².

BROADWAY, D.C.; GRIERSON, I.; STÜRMER, J.; HITCHINGS, R.A. Reversal of topical antiglaucoma medication effects on the conjunctiva. **Archives of Ophthalmology**.v.114, n., p.262-267, 1996.

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**. v.79, n.3, p. 309-312, 2001.

DE SAINT JEAN, M.; BRIGNOLE, F.; BRINGUIER, A. F.; BAUCHET, A.; FELDMANN, G.; BAUDOUIN, C. Effects of benzalkonium chloride on growth and survival of Chang conjunctival cells. **Investigative Ophthalmology Visual Science**. v.40, n.3, p. 619-630, 1999.

GORDON, M.O.; BEISER, J.A.; BRANDT, J.D.; HEUER, D.K.; HIGGINBOTHAM, E. J.; JOHNSON, C. A.; et al. The Ocular Hypertension Treatment Study: Baseline factors that predict the onset of primary open-angle glaucoma. **Archives of Ophthalmology**. v.120, n.6, p. 714-720, 2002. Comment in: **Archives of Ophthalmology**. v.122, n.7, p.1088-1089, author reply 1089, 2004.

FARIA N.V.L., RUSS H.H, 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. **Journal of Ophthalmic Inflammation and Infection**, 3:22, 2013.

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**. v.113, n.6, p.930-936, 2006.

FRANCIS, B. A.; DU, L. T.; NAJAFI, K.; MURTHY, R.; KURUMETY, U.; RAO, N.; MINCKLER, D. S. Histopathologic features of conjunctival filtering blebs. **Archives of Ophthalmology**. v.123, n.2, p.166-170, 2005.

HERRERAS, J. M.; PASTOR, J. C.; CALONGE, M.; ASENSIO, V. M. Ocular surface alteration after long-term treatment with antiglaucomatous drug. **Ophthalmology**. v.99, n.7, p.1082-1088, 1992. Comment in: **Ophthalmology**. v.100, n.3, p.293-294, 1993.

KUNERT, K. S.; TISDALE, A. S.; GIPSON, H. K. Goblet cell number and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine. **Archives of Ophthalmology**. v.120, n.3, p.330-337, 2002.

LAMA, P. J.; FECHTNER, R. D. Antifibrotics and wound healing in glaucoma surgery. **Survey of Ophthalmology**. v.48, n.3, p.314-346, 2003.

LAVIN, M. J.; WORMALD, R. P.; MIGDAL, C. S.; HITCHINGS, R. A. The influence of prior therapy on the success of trabeculectomy. **Archives of Ophthalmology**. v.108, n.11, p.1543-1548, 1990.

LEONARDI, A.; DEFRANCHIS, G.; FREGONA, I. A.; VIOLATO, D.; PLEBANI, M.; SECCHI, A. G. Effects of cyclosporin A on human conjunctival fibroblasts. **Archives of Ophthalmology**. v.119, n.10, p.1512-1517, 2001.

MOURA, C. R.; MELLO, P. A. A. Tratamiento quirúrgico de glaucoma. In.: MELLO, P.A. A. **Glaucoma**. 1a. ed, Quito: Nocion, 1999, p.201-221.

MIETZ, H.; NIESEN, U.; KRIEGLSTEIN, G. K. The effect of preservatives and antiglaucomatous medication on the histopathology of the conjunctiva. **Graefe's Archive for Clinical and Experimental Ophthalmology**. v.232, n.9, p.561-565, 1994.

MIETZ, H.; JACOBI, P.C.; WELSANDT, G.; KRIEGLSTEIN, G. K. Trabeculectomies in fellow eyes have an increase risk of Tenon's capsule cysts. **Ophthalmology**. v.109, n.5, p.992-997, 2002.

NGUYEN, K. D.; LEE, D. A. Effects of steroids and nonsteroidal antiinflammatory agents on human ocular fibroblast. **Investigative Ophthalmology & Visual Science**. v.33, p.2693-2701, 1992.

PFEIFFER, N.; GRIESERSON, 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**. v.119, n.2, p.191-196, 2001.

RITCH, R.; SHIELDS, B.; KRUPIN, T. Pharmacology. In: RITCH R, SHIELDS MB, KRUPIN T. **The glaucomas**. 2ª ed. St. Louis: Mosby, 1996, vol.3, p.1375-1489.

RUSS, H. H.; COSTA, V. P.; FERREIRA, F. M.; VALGAS, S. R.; CORREA NETO, M.A.; STROBEL, E. L.; TRUPPEL, J. H. Conjunctival Changes Induced by Prostaglandin Analogues and Timolol Maleate: A Histomorphometric Study. **Arquivos Brasileiros de Oftalmologia**. v. 70, n.6, p.910-916, 2007.

SHERWOOD, M. B.; GRIERSON, I.; MILLAR, L.; HITCHINGS, R. A. Long-term morphologic effects of antiglaucoma drugs on the conjunctiva and Tenon's capsule in glaucomatous patients. **Ophthalmology**. v.96, n.3, p.327-335, 1989.

SUSANNA JUNIOR, R.; MEDEIROS, F. A. Trabeculectomia. In: SUSANNA JUNIOR, R. **Cirurgia do Glaucoma**. 2ª ed. São Paulo: Rocca, 2002, p.27-64.

WEINREB, R. N.; MEDEIROS, F. A. Como **definir** o glaucoma? In: SUSANNA JUNIOR, R.; WEINREB, R. N. **Glaucoma: Perguntas e Respostas**. 1ª ed. Rio de Janeiro: Cultura Médica, 2005, p.1-7.

TURACLI, M. E.; GUNDUZ, K.; AKTAN, G.; SENCER, H. Topical cyclosporine as a possible new antimetabolite in trabeculectomy. **Ophthalmic Surgery & Lasers**. v.27, n.6, p.438-444, 1996.

| TURACLI, M. E.; GUNDUZ, K.; AKTAN, G.; TAMER, C. A comparative clinical trial of mitomycin C and cyclosporine A in trabeculectomy. **European Journal of Ophthalmology**.v.6, n.4, p.398-401, 1996.

VIJAVA, L.; MUKHESH, B. N.; SHANTA, B.; RAMALINGAM, S.; SATHI DEVI, A. V. Comparison of lowdose intraoperative mitomycin-C vs 5-fluorouracil in primary glaucoma surgery: a pilot study. **Ophthalmic Surgery & Lasers**.v.31, n.1, p.24-30, 2000.

WUDUNN, D.; CANTOR, L. B.; PALANCA-CAPISTRANO, A. M.; HOOP, J.; ALVI, N. P.; FINLEV, C.; LAKHANI, V.; BURNSTEIN, A.; KNOTTS, S. L. Long-term outcomes of intraoperative 5-fluorouracil versus intraoperative mitomycin C in primary trabeculectomy surgery. **American Journal of Ophthalmology**.v.134, n.4, p.521-528, 2002.

YOUNG, L.T.; HIGGINBOTHAM, E. J.; ZOU, X. L.; FARBER, M. D. Effects of topical glaucoma drugs on fistulized rabbit conjunctiva. **Ophthalmology**. v.97, n.11, p.1423-1427, 1990.