INTRA-OCULAR PRESSURE IN NEW ZEALAND RABBIT AFTER TEFLON TUBE IMPLANT ASSOCIATED TO MITOMYCIN C

PRESSÃO INTRA-OCULAR APÓS IMPLANTE DE TUBO DE TEFLON ASSOCIADO À MITOMICINA C EM COELHOS DA RAÇA NOVA ZELÂNDIA

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SUMMARY

Fourteen healthy, adult New Zealand rabbits, males and females, were used to evaluate the histological and clinical aspects after the implant of Teflon tube associated to mytomicin C. The ten right eyes of group I and ten left eyes of group II were evaluated for 30 and 60 days of postoperative (PO), respectively. The other eight eyes of group III were observed for 48 hours. A bag was constituted in the bulbar conjunctive and inside of it mytomicin C was applied. A Teflon tube was introduced in the anterior chamber and fastened in the sclera, the conjunctive bag was approximated in the limbus over the implant. There was significant difference in the averages of the intraocular pressures (IOP) between the preoperative and the postoperative of 30 and 60 days. The animals of groups I and II presented hypotonia and shallow anterior chamber in the first 15 days of PO. In the histological evaluation of the conjunctive bag, descaling of the epithelium superficial cells and degeneration of the conjunctive tissue was verified, which indicated healing changes due to the action of mytomicin C. The implant of Teflon associated with mytomicin C demonstrated to be an effective method to decrease and to maintain the IOP from 30 to 60 days PO.

KEY-WORDS: Antifibroblasts. Aqueous Humor. Glaucoma. Filtrative Surgery. Ophthalmology. Oryctolagus cuniculus.

RESUMO

Foram utilizados 14 coelhos da raça Nova Zelândia, adultos, hígidos, machos e fêmeas, para avaliar os aspectos histológicos e clínicos após o implante de tubo de teflon associado a mitomicina C. Dez olhos direitos formaram o grupo I e dez olhos esquerdos formaram o grupo II avaliados por 30 e 60 dias de pós-operatório (PO), respectivamente. Os outros oito olhos formaram o grupo III observados por 48 horas. Foi constituído uma bolsa na conjuntiva bulbar e dentro dela foi aplicado mitomicina C. Um tubo de teflon foi introduzido na câmara anterior e fixado na esclera, a bolsa conjuntival foi aproximada no limbo sobre o implante. Houve diferença significativa nas médias das pressões intra-oculares (PIO) entre o pré-operatório e o pós-operatório de 30 e 60 dias. Os animais dos grupos I e II apresentaram hipotonia e câmara anterior rasa nos primeiros 15 dias de PO. Na avaliação histológica da bolsa conjuntival foi verificado descamação das células superficiais do epitélio e degeneração do tecido conjuntivo, o que indicou alterações na cicatrização mediante ação da mitomicina C. O implante de teflon associado com mitomicina C demonstrou ser um método eficaz por diminuir e manter a PIO dos 30 aos 60 dias de PO.

PALAVRAS-CHAVE: Antifibroblásticos. Cirurgia Filtrativa. Glaucoma. Humor Aquoso. Oftalmologia. Oryctolagus cuniculus.

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INTRODUCTION

Glaucoma is a serious eye disease that can be considered an ophthalmic emergency in small animals. It is an optic neuropathy, which may cause damage to the retinal ganglion cells and optic nerve axons, leading to loss of vision (GELATT, 2003). It is characterized by the increase of intraocular pressure (IOP) resulting from excess aqueous humor circulating in the anterior and posterior chambers of the eyeball (EURIDES & SILVA, 2013).

The surgeries for controlling IOP are classified as procedures that reduce the formation of aqueous humor and techniques that increase the fluid drainage such as trabeculectomy with or without deep sclerectomy, iridencleisis, cyclodyalisis (EURIDES, 2004) and synthetic implants (GELATT, 2003). Several implants have been used in human and veterinary medicine such as stellon (MOLTENO, 1969), schocket (KIM & HWANG, 1988), kuprin drain (OZDAMAR et al., 2003), ahmed (SAPIENZA & WOERDT, 2005), gonioseton (VAN DER VEEN et al., 1990), express (FREEDMAN & TROPE, 2005) and polymethylmethacrylate laminar implant (JACOBOVITZ, 2004).

The healing of draining fistulas and implant obstruction with inflammatory debris and scar tissue are the main causes of failure of antiglaucoma filtration procedures (SOARES et al., 2005). The use of antifibroblastics such as mitomycin C (MMC) during surgery has been studied, aiming to reduce the formation of collagen by fibroblasts in the proliferative phase of wound healing (LAMA & FECHTNER, 2003).

This study analyzes the histological aspects of the bulbar conjunctiva and sclera, and evaluates the clinical aspects of the eye, 30 and 60 days after implantation of Teflon tube in the anterior chamber associated with topical application of subconjunctival mitomycin C in healthy rabbits.

MATERIAL AND METHODS

We used 14 healthy New Zealand rabbits, males and females, with 19.10 ± 1.2 mmHg mean IOP and 2.4 ± 0.3 kg average weight. The animals were housed in individual cages and fed comercial diet[6] and water ad libitum.

The ten right eyes of group I and ten left eyes of group II were evaluated for 30 and 60 postoperative (PO) days, respectively. The eight eyes of the remaining four rabbits formed group III and were observed for 48 hours.

After desensitization of the corneas with anesthetic eye drops containing oxibuprocaine[7], the Tonopen[8] tonometer was used to measure the intraocular pressure of the right and left eyes of rabbits, 24 hours before the surgery and weekly, at 2pm, during 30 and 60 days, postoperatively. The comparing value of pressures of rabbits was 15-20 mmHg (HERRERA, 2008).

Preoperatively, the animals abstained from water and solid food for six hours while enrofloxacin[9] (10.0 mg/kg/SC), Tramadol[10] (2.0 mg/kg/SC) and ketoprofen[11] (2.0 mg/kg) were administered 20 minutes prior to the surgical procedure. The premedication was made with acepromazine[12] (0.1 mg/kg/IM) whereas anesthesia was induced and maintained with ketamine[13] (5.0 mg/kg/IM) and xylazine[14] (1.0 mg/kg/IM).

A portion of 8.0 mm was removed from the pointed end of a 25.0-mm (22G) fluorinated ethylene propylene polymer (Teflon) catheter[15], with 0.9 mm diameter. A blade[16] was used to cut longitudinally, opposite the pointed end, two 3.0-mm long flanges.

The animal was positioned in lateral decubitus and the eyeball was immobilized in the medial and lateral corners with two separated single stitches using 8-0 nylon suture thread[17]. After two hours, under an operating microscope[18] and using scissors a 10.0 x 7.0 mm pocket was made, from the corneal limbus in the bulbar conjunctiva, of approximately 10.0 mm diameter. A wad of cotton soaked with mitomycin C[19] (0.5 mg/mL) was placed inside the pocket for three minutes (Figure 1A). The cotton was removed and the conjunctival pocket was irrigated with approximately 50 mL of ringer lactate solution[20]. The 0.9-mm diameter catheter needle was used to pierce a 2.0-mm hole in the sclera from the corneal limbus into the anterior chamber. The Teflon tube was introduced through the hole, leaving a 5.0-mm extension in the anterior chamber while the flanges were fixed on the sclera with simple and separate suture stitches using Polyglactin 910 7-0[21] (Figure 1B). The implant was covered with a conjunctival pocket and approached to the corneal limbus with separated simple suture and lying U pattern with Polyglactin 910 7-0. The volume of 1 mL of 0.005% trypan blue[22] stain was injected into the anterior chamber through the limbus to check the drainage of aqueous humor into the pocket immediately after surgery and, 30 and 60 postoperative days, as well (Figure 1C).

[6]Sítio Coelhão. Guabi. Campinas, SP. Brasil.
[7]Oxinest. Latinofarma. Cotia, SP. Brasil.
[8]Tono-Pen XL. Medtronic Solan. Jacksonville, FL. USA.
[9]Quinotril. Valle Produtos Veterinários. Montes Claros, MG. Brasil.
[10]Tramal. União Química Farmacêutica Nacional. Pouso Alegre, MG. Brasil.
[11]Ketojet. União Química Farmacêutica Nacional. Guaçu, SP. Brasil.
[12]Acepran. Univet. São Paulo, SP. Brasil.
[13]Cetamin. Syntec. Patrocínio Paulista, SP. Brasil.
[14]Kensol. Kong. Santana de Parnaíba, SP. Brasil.
[15]BD Angiocath. Becton Dickinson. Juiz de Fora, MG. Brasil.
[16]Laser Platinum. Rimed. São Paulo, SP. Brasil.
[17]Nylon 8-0. Polysuture Indústria e Comércio. São Sebastião do Paraíso, MG. Brasil.
[18]1902. D.F. Vasconcellos S.A. São Paulo, SP. Brasil.
[19]Mitocin. Bristol-Myers Squibb. São Paulo, SP. Brasil.
[20]Ringer com Lactato de Sódio. Aster. Sorocaba, SP. Brasil.
[21]Vicryl 7-0. Ethicon. São Paulo, SP. Brasil.
[22]Bluepoint. Oftalmopharma. Artur Nogueira, SP. Brasil.

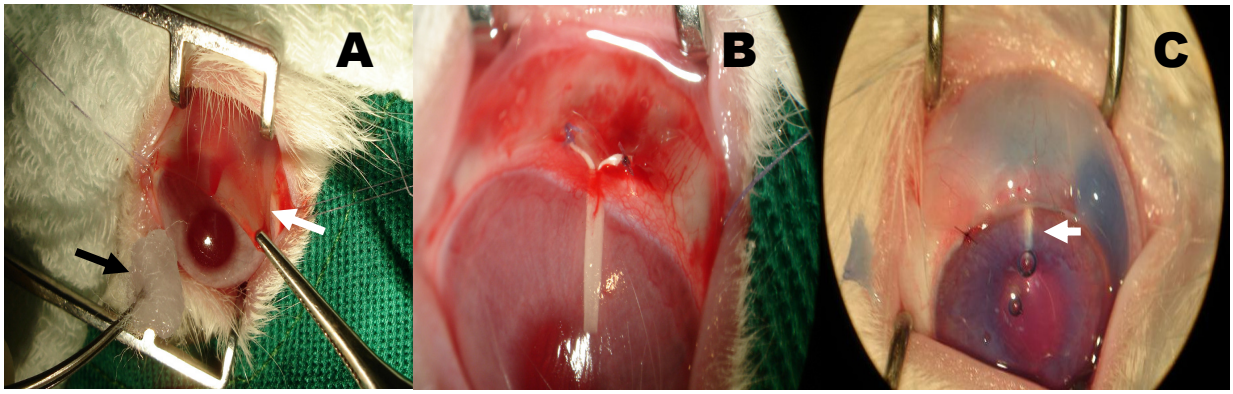

Figure 1 – Implant of a drainage teflon tube associated with mitomycin C in New Zeland rabbit. Observe a cotton swab soaked with mitomycin C (black arrow) being introduced into the conjunctival pocket (white arrow) (A). Teflon tube inserted into the anterior chamber. Observe the tube flanges mounted on the sclera with separated single stitches using Polyglactin 910 7-0 (B). Conjunctival pocket attached to the corneal limbus on the tube (arrow). Note the presence of trypan blue dye into the anterior chamber and conjunctival pocket (C).

Topical ointment containing 0.1% dexamethasone, 0.35% neomycin and polymyxin B 6000UI[23] and 1% atropine sulfate eye drops[24] were applied every eight hours for seven days, postoperatively.

After the pre-determined postoperative periods, the rabbits were euthanized for collection of the ocular bulbs with an overdose of thiopental sodium 2.5%[25] and potassium chloride 10%[26], as recommended by the code of ethics for the use of animals in scientific research (AMERICAN VETERINARY MEDICAL ASSOCIATION, 2001). The material was fixed in 10% formalin[27], cut and embedded in paraffin. The histological sections were stained using hematoxylin and eosin (HE) and picro-sirius red counterstained with fast green. The slides were analyzed using optical microscope[28] images for verification of tissue reactions and scarring of the conjunctival pocket and the region around the hole where the Teflon tube was implanted.

RESULTS

The mean IOP preoperative values of the right eyes were compared quantitatively at 7, 14, 21 and 30 and of the left eyes at 7, 14, 21, 30, 37, 45, 52 and 60 postoperative days. Data were subjected to analysis of variance for randomized block design and means were compared using the Scott-Knott test at 5% significance level, in the SISVAR software (FERREIRA, 2000). Analysis of variance was significant ($p \leq 0.05$) in mean IOP between the preoperative (19.58 ± 0.88 mmHg and 20.10 ± 1.2 mmHg for right and left eyes, respectively) and post-operatively at 30 (9.12 ± 0.51 mmHg) and 60 days (9.74 ± 2.6 mm Hg), Figure 2.

The animals in groups I and II showed hypotonia with mean IOP of 4.0 mmHg and shallow anterior chamber in the first two weeks, postoperatively. From the third week, the IOP had an average value of 9.0 mmHg and deepening of the anterior chamber.

Histological evaluation of conjunctival pocket of group III, two days after surgery, showed intense desquamation of superficial epithelial cells, with the presence of the basal layer only and discrete signs of degeneration of the collagen fibers of the connective tissue (Figure 3A) .

At 30 days, postoperatively, the conjunctival pocket showed degeneration signs of the collagen fibers of the connective tissue, absence of blood vessels and mucus-secreting cells. Rabbits of group I also displayed severe desquamation of the superficial epithelial cells, leaving only the basal layer (Figure 3B).

At 60 days postoperatively, the pocket had atypical healing process, with intense neovascularization and existence of fibrocystic hyperplasia. The epithelium was stratified in all its layers with the presence of mucus-secreting cells (Figure 3C).

[23]Maxitrol. Alcon Laboratórios do Brasil. São Paulo, SP. Brasil.
[24]Atropina 1%. Allergan. Guarulhos, SP. Brasil.
[25]Tio Pental. Cristália Produtos Químicos Farmacêuticos. Campinas, SP. Brasil.
[26]Cloreto de Potássio 10%. Darrow Laboratórios S.A. Rio de Janeiro, RJ. Brasil.
[27]Formol. Start Química. Uberlândia, MG. Brasil.
[28]BX 40. Olympus Shinjuku-ku. Tóquio, Honshu. Japan.

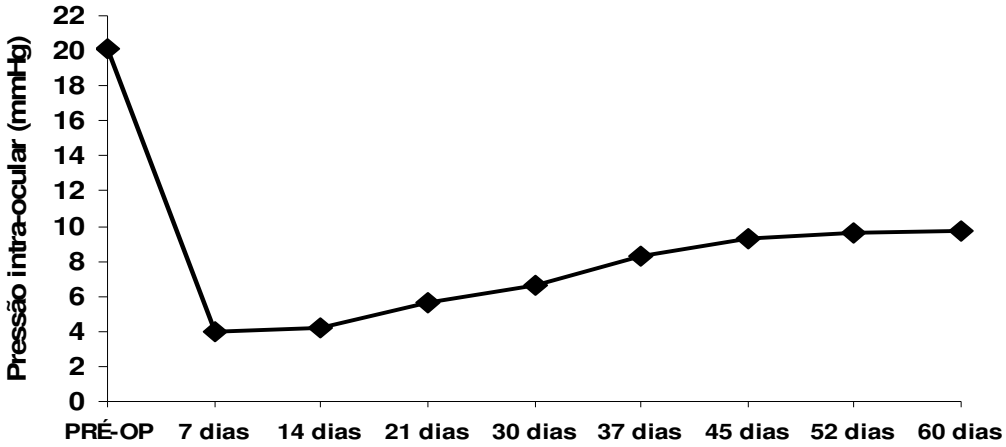

Figure 2 - Mean values of intraocular pressure (mmHg) preoperatively (PRE-OP) and 7, 14, 21, 30, 37, 45, 52 and 60 days after implantation of the Teflon tube in the anterior chamber with subconjunctival mitomycin C, in New Zealand rabbits.

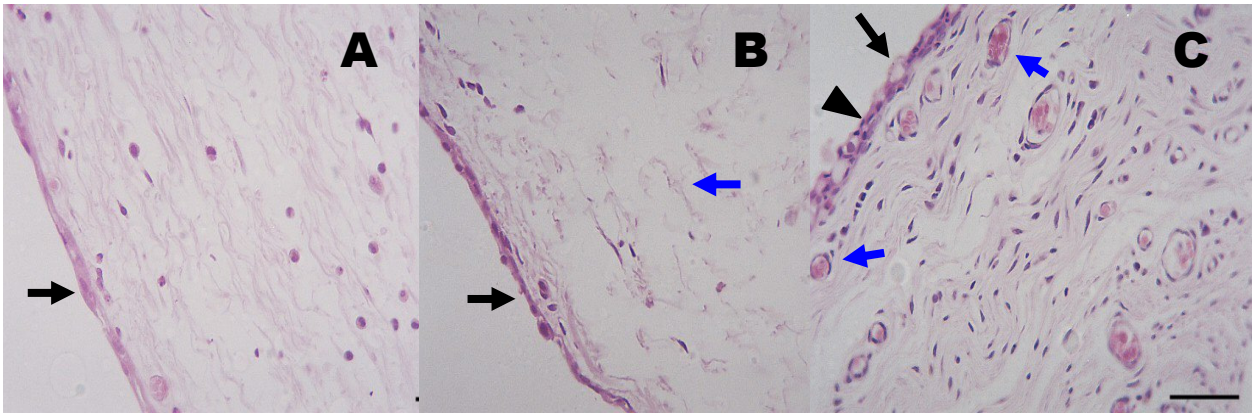

Figure 3 - Photomicrograph of conjunctival pocket in New Zealand rabbits at 48 hours (A), 30 (B) and 60 days (C) PO. Observe the existence of the basal layer only (black arrow) and degeneration of the collagen fibers of the connective tissue (A). Absence of blood vessels and mucus-secreting cells, degeneration of collagen fibers (blue arrow) and the existence of the basal layer only (black arrow) (B). Fibrocystic hyperplasia, mucus-secreting cells (black arrow), neovascularization (blue arrow) and stratified epithelium (arrowhead) (C). HE (bar = 100µm).

DISCUSSION

The data obtained after analysis of variance for randomized block design indicate that implantation of Teflon tube remained well stabilized and clear, allowing the drainage of aqueous humor into the conjunctival pocket.

Teflon being a flexible material was easily introduced into the anterior chamber of rabbits in this study. It does not cause undesirable reactions to the body when used aseptically (MARASSI & FERREIRA, 2005). Tubular implants have been tested for drainage of aqueous humor in rabbits (MOLTENO, 1969) and humans (OZDAMAR et al., 2003). The choice of a Teflon catheter for making the implant of this study was due to the tubular shape that enabled to pass it through the orifice made in the sclera with the catheter needle.

The suture thread was chosen because it stimulates little proliferation of connective tissue (EURIDES, 2004), sufficient to keep the tube and the conjunctival pocket fixed for up to 60 days. Implants loosely attached to the sclera can stimulate inflammation in the surrounding tissue (MOLTENO, 1969). Optical microscopy analysis showed mild connective tissue formation at the site of the tube suture.

Hypotonia reported in this study can be explained due to drainage of aqueous humor through the Teflon tube and the hole around the implant, which did not have signs of healing as observed histologically at 48 hours after surgery. At 30 days, postoperatively, a capsule with thin collagen fibers interspersed with fibrocytes was noted around the tube. Possibly, the aqueous humor no longer overflowed the hole confined to the tube, which justified the increase in IOP from 4.0 to 9.0 mmHg in the third week postoperatively. Therefore, it is possible that the healing process around the implant has started from 15 days after surgery.

Histological data similar to those of group III (two postoperative days) of this experiment, were found by Bergstrom et al. (1991) and Holzchuh et al. (2004), who stated that the desquamation of the epithelial cell is reduced by the antiproliferative effect of MMC at concentrations of 0.5 mg/mL and 0.2 mg/ml, respectively. The basal layer is formed by mitotically active cells responsible for the constant renewal of the epithelium, which gives rise to other epithelial layers (GARTNER & HIATT, 1999). In this work, only the basal layer was noticed, which indicated a decrease of mitotic activity due to the action of mitomycin C (LAMA & FECHTNER, 2003).

In the proliferative phase of scaring, macrophages stimulate the proliferation of fibroblasts leading to collagen (WOUK et al., 1999). As MMC is an antifibroblastic drug that causes vascular changes and hypoxia (BERGSTROM et al., 1991), few collagen fibers are produced and in altered conditions, which explains the degeneration of collagen fibers observed in group I (30 days postoperatively). The absence of blood vessels by the action of MMC (MATAYOSHI et al., 2003) was also confirmed in ocular surgery by Bergstrom et al. (1991), Wouk et al. (1999).

The absence of mucus-secreting cells at 30 PO days can be justified by the deleterious action of MMC on the goblet cells (HOLZCHUH et al., 2004). Despite the absence of mucus-secreting cells in the conjunctiva of the pocket, no clinical signs of keratoconjunctivitis sicca were observed. This fact is due to the action of these cells located in other parts of the bulbar and palpebral conjunctiva, which were not treated with mitomycin C.

The presence only of the basal layer in group I (30 days postoperatively), indicated decreased mitotic activity by the action of MMC, as observed at two days postoperatively.

Histological findings in group II (60 days postoperatively) indicate that the healing evolved throughout the first 30 days, but it became ineffective for the period of 60 days postoperatively.

CONCLUSION

Clinical and histological observations of the Teflon tube implant for drainage of aqueous humor in healthy New Zealand rabbits proved to be a safe and effective method for reducing and maintaining the intraocular pressure (9.74 mmHg) from 30 to 60 days PO. The associated mitomycin C at a concentration of 0.5 mg/ml was effective for delaying the healing of the conjunctival pocket and preventing the clogging of the Teflon tube during the observation period.

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