Ciência Animal Brasileira

DOI: 10.1590/1809-6891v21e-57717

Intracameral tenecteplase during phacoemulsification in rabbits: clinical assessment of the anterior segment and biochemical analysis of the aqueous humor

Tenecteplase intracameral durante a facoemulsificação em coelhos: avaliação clínica do segmento anterior e análise bioquímica do humor aquoso

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Section: Medicina Veterinária

Received
March 17, 2019.
Accepted
December 4, 2019.
Published
June 16, 2020.

www.revistas.ufg.br/vet
visit the website to get the how to cite in the article page.

Abstract
To evaluate the use of tenecteplase in transoperative phacoemulsification in healthy rabbits, the study was carried out with fifteen New Zealand rabbits, divided into three groups: control group (CG), untreated group (UG) and treated group (TG). UG and TG were operated by phacoemulsification and TG received 50 µg / 0.3 mL of intracameral tenecteplase. The postoperative evaluations were 24 h, 72 h, 7 days, 15 days and 21 days. In TP21 the animals were submitted to euthanasia and aqueous humor samples were collected. No significant differences were observed in the clinical evaluations between CG and TG in relation to incidence rates of intraocular pressure (IOP), corneal edema, fibrin deposits, hyphema, aqueous flare and synechia. In the physicochemical evaluation of the aqueous humor, there were no significant differences between the three groups in relation to pH values and concentrations of chloride ions. The aqueous humor density values were statistically different between CG and the other groups. In the histological evaluation, there were no significant differences between the groups. The use of tenecteplase in transoperative phacoemulsification in rabbits did not present significant differences in terms of clinical, physicochemical and histological parameters.
Keywords: cataract, fibrin, ocular inflammation, *Oryctolagus cuniculus*, phacoemulsication, tenecteplase.

Resumo
Para avaliar o uso da tenecteplase na facoemulsificação transoperatória em coelhos sadios, o estudo foi realizado com quinze coelhos da raça Nova Zelândia, divididos em três grupos: grupo controle (CG) grupo não tratado (UG) e grupo tratado (TG). UG e TG foram operados por facoemulsificação e TG recebeu 50 µg / 0,3 mL de tenecteplase intracameral. As avaliações pós-operatórias foram 24 h, 72 h, 7 dias, 15 dias e 21 dias. No TP21 os animais foram submetidos à eutanásia e amostras de humor aquoso foram coletadas. Não foram observadas diferenças significativas nas avaliações clínicas entre o CG e o TG em relação às taxas de incidência de

pressão intraocular (PIO), edema de córnea, depósitos de fibrina, hifema, flare aquoso e sinéquia. Na avaliação físico-química do humor aquoso, não houve diferenças significativas entre os três grupos em relação aos valores de pH e concentrações de íons cloreto. Os valores de densidade de humor aquoso foram estatisticamente diferentes entre CG e os outros grupos. Na avaliação histológica, não houve diferenças significativas entre os grupos. O uso da tenecteplase na facoemulsificação transoperatória em coelho não apresentou diferenças significativas em termos de parâmetros clínicos, físico-químicos e histológicos.
Palavras-chave: catarata, fibrina, inflamação ocular, *Oryctolagus cuniculus*, facoemulsificação, tenecteplase.

Introduction

Cataracts is one of the main causes of vision loss in humans and domestic animals[1]. Removal of the opaque lens is the single effective treatment, with phacoemulsification (PHACO) being the most widely used technique[1,2]. Uveitis stands out among the postoperative complications following PHACO and is due to the immune response to the presence of lenticular proteins in the anterior chamber (AC) and to tissue injury resulting from excessive intraoperative manipulation of the intraocular structures. Disruption of the blood-aqueous barrier (BAB), which might be due to both surgical manipulation and uveitis, results in cell migration and extravasation of proteins from the vascular compartment to the AC, where they promote changes in the composition of the aqueous humor (AH)[3,4]. Fibrin deposition in the AH has been observed in the eyes of dogs/humans with anterior uveitis[5,6] and is directly related to the postoperative period following PHACO[5,7]. The presence of fibrin in the AC is a predisposing factor for the occurrence of synechia, pupillary block, secondary glaucoma and blindness[3,4,8].

Several therapeutic protocols have been suggested in the attempt to avoid or minimize fibrin deposition in the AC and its complications, including preservative-free 1% lidocaine, heparin, indomethacin, fluorouracil, colchicine, methotrexate, selective COX-2 inhibitors, such as celecoxib and rofecoxib[7], and tissue plasminogen activators (TPAs), the latter being the drugs most widely employed[6].

Synthetic TPAs are proteases with fibrinolytic activity, acting on the conversion of plasminogen to plasmin, which is responsible for the degradation of fibrin[5]. TPAs are used by intravenous route for treatment of thromboembolic myocardial infarction in humans. When administered the intraocular route, they also manifest their capacity to cleave fibrin and dissolve clots, without causing damage to the corneal endothelium[9,10]. In humans, TPAs are among the first-choice drugs to dissolve intraocular fibrin[11] and treat hyphema[10] and are also used for the treatment of subretinal hemorrhage, vitreous liquefaction and posterior vitreous detachment[12]. In veterinary medicine, these agents have been transoperatively employed during lens extraction[13], for the dissolution of fibrin deposits, treatment of hyphema and in the postoperative period of intraocular surgery[8,10].

Tenecteplase (TNK) is a third-generation TPA, has a 6-fold prolonged plasma half-life, 15-fold higher fibrin specificity, and 80-fold reduced binding affinity to physiological plasminogen activator inhibitor (PAI-1)[14,15]. In addition, the TNK vehicle contains lesser L-arginine (below one-third of the tPA) probably the cause of toxicity in the posterior segment[13,15,16]. However, more study is needed regarding the use of TNK in association with PHACO. For this reason, the aim of the present research was to evaluate the transoperative use of TNK during PHACO in healthy rabbits and to describe aspects related to clinical parameters and the physicochemical assessment of AH.

Materials and methods

The present study was approved by the ethical committee for animal use (Comissão de Ética no Uso de Animais - CEUA) of the Federal University of Goias (Universidade Federal de Goiás - UFG), protocol no. 022/15, and complied with the guidelines for animal experimentation formulated by the *Association for Research in Vision and Ophthalmology* (ARVO).

TNK (Metalyse®, Boehringer-Ingelhein, Itapecerica da Serra/São Paulo (SP)/Brazil) was processed in a laminar flow hood via dilution with balanced saline solution (BSS) until a concentration of 0.05% (50µg) was achieved. The solution was divided into 0.1-ml aliquots, which were stored in micro tubes in a freezer at -80 ºC[15]. The samples were removed from the freezer on the day of the surgical procedure and were kept in a cooler at -20ºC until use, when they were thawed at room temperature.

Thirty eyes were used from 15 male albino New Zealand rabbits with age 90 to 160 days and weight 2.5-3 kg. The animals were kept in individual cages at the Animal Experimentation Unit, School of Veterinary Medicine and Animal Science (Escola de Veterinária e Zootecnia - EVZ/UFG), with free access to food and water, a 12-hour light cycle and a temperature of 24 °C.

Before the onset of the experiment, the animals were subjected to clinical, ophthalmologic and hematologic examinations to certify their general and ocular health. The eye examination consisted of the following sequence, always performed by the same evaluator: Schirmer's test, neuroophthalmologic evaluation (threat response, glare test, eyelid reflex, direct and consensual photopupillary reflex), fluorescein eye stain, tonometry (Tono-Pen Avia® Applanation-Reichert, Buffalo, Nova Iorque, EUA), slit light examination and indirect ophthalmoscopy. This sequence of the ophthalmic examination was performed on the seven days before the PHACO (time point - TP 0) and one (TP1), three (TP3), seven (TP7), 15 (TO 15) and 21 (TP 21) days after the PHACO.

Twenty-four eyes from 12 rabbits were subjected to PHACO and were randomly allocated to the following groups: an untreated group (UG), which did not receive TNK, and a treated group (TG), which received intracameral TNK. Six eyes from three rabbits not subjected to PHACO served as a control group (CG) for the assessment of the physicochemical parameters of AH.

The animals underwent an adaptation period of seven days, with Elizabethan collar,

before surgery, performed without previous fasting. All anesthetic and surgical procedures were performed by the same team. Pharmacological mydriasis was induced with 1% tropicamide eye drops (Mydriacyl®, Alcon, São Paulo/SP/Brazil) and 10% epinephrine eye drops (Fenilefrina 10%®, Allergan, Guarulhos/SP/Brazil)[1,4]. Pre-anesthetic medications included morphine 2 mg/kg and ketamine 10 mg/kg combined with xylazine 5 mg/kg was administered per the intramuscular (IM) route. Induction was performed with propofol 2 mg/kg per the intravenous (IV) route, followed by intubation using no. 3 orotracheal tubes and maintenance by means of isoflurane in an open circuit. Next, 0.5% proxymethacaine eye drops (Anestalcon®, Alcon, São Paulo/SP/Brazil) were instilled.

The main and accessory incisions were performed according to the clear corneal cataract surgery. The procedure began by the accessory incision, which was performed with a 15-degree scalpel at 2-3 o'clock. A total of 0.2 ml of 0.1% of trypan blue (Ophthalmus, São Paulo/SP/Brazil), balanced salt solution (BSS) and 2% methylcellulose (Ophthalmus, São Paulo/SP/Brasil) were applied into the AC. Next, the main incision was performed with a 3.2-mm scalpel at 9-10 o'clock. Capsulorhexis was performed using a cystotome and Utrata forceps. After BSS hydrodissection and rotation of the lens nucleus with the phacoemulsifier probe (6) (Phaco XL®, Staar, Monrovia, California, USA), the lens nucleus was only aspirated and irrigated until a cortex ring was obtained in the pouch capsular. The cortex ring was aspired using a two-way cannula. Next, the main incision was closed by means of two simple interrupted 9-0 nylon sutures[1,6]. The animals from the TG were administered 0.1 ml of 0.05% TNK solution into the AC, and the ones from the UG were administered 0.1 ml of BSS immediately after suturing.

All of the animals' eyes received one single application of gentamycin 20 mg combined with dexamethasone 1 mg per the subconjunctival route and terramycin ophthalmic ointment in the immediate postoperative period. Postoperative analgesia was achieved by means of tramadol hydrochloride 4 mg/kg per the subcutaneous (SC) route three times a day (TID), one single dose of meloxicam 0.2 mg/kg and two doses of 0.1 mg/kg of meloxicam once a day (QD) per the SC route. All of the animals received one drop of 1% tropicamide eye drop TID[8].

The animals' eyes were assessed seven days before (time-point - TP_0) and one (TP_1), three (TP_3), seven (TP_7), 15 (TP_{15}) and 21 (TP_{21}) days after PHACO.

The cornea was assessed as to the presence of diffuse opacity according to the scores described by Eaton et al.[17]. For the previous camera (AC), Hogan's aqueous flare score[18], fibrin score[19] and the presence or absence of hyphema were considered. The iris was assessed regarding inflammation, which was analyzed based on a score adapted from the one formulated by Eaton et al.[17] along with the presence or absence of synechia (Table 1).

Euthanasia was performed on TP_{21} with tiopental 150 mg/kg (Tiopental sódico®, Cristália Prod. Quim. Farm. Ltda., São Paulo/SP/Brazil) and 10 ml of 19,1% potassium chloride IV (Equiplex Ind. Quim & Farm. Ltda, Aparecida de Goiânia/Goias (GO)/Brazil).

Following euthanasia, the eyeballs were removed. Next, 0,3 ml of AH was collected

through paracentesis, transferred to a polypropylene vial containing EDTA and divided in two 0,15ml aliquots for physical and biochemical analysis. The eyeballs were then fixed with 10% buffered formalin for 24 hours and transferred to 70% ethanol solution, where they were kept until histological processing.

Table 1. Scores of diffuse corneal opacity, aqueous flare, fibrin and hyphema in the anterior chamber and iris inflammation[17,18,19]

Parameters	Score	Description
Corneal opacity	0	Absent
	1	Discrete, good visualization of iris details
	2	Moderate, reduced visualization of iris details
	3	Severe, iris details not visible
Aqueous flare	0	Absent
	1	Discrete, good visualization of iris details
	2	Moderate, reduced visualization of iris details
	3	Severe, iris details not visible
Fibrin	0	Absent
	1	Discrete, affects only 1 quadrant of the pupillary
	2	Moderate, affects 2 to 3 quadrants of the pupillary
	3	Severe, affects the entire pupillary margin
Hyphema	+	Present
	-	Absent
Iris inflammation	0	Absent
	1	Discrete, with focal hyperemia, without changes in the
	2	Moderate, with diffuse hyperemia and reduced red
	3	Severe, with diffuse hyperemia and loss of the red
Synechia	+	Present
	-	Absent

The AH density was assessed by means of refractometry (Ellman BIO 2000®, Bioplus, Barueri/SP/Brazil) and the pH with test strips. The concentrations of total proteins and chloride ions were measured by means of colorimetric testing using an automatic biochemical analyzer (CM 200®, Wiener lab Group, Rosário, Argentina).

The eyeballs were processed to obtain histological slides stained using the hematoxylin-eosin technique. The structures assessed were the iris and ciliary body. The parameters analyzed were presence or absence of adhesions, fibrin, fibrosis and hemorrhage.

The data were processed using BioStat 5.0 software (IDMS, Belem, Brazil). Intraocular pressure (IOP), corneal opacity, aqueous flare, fibrin in the AC and iris inflammation was compared between groups and time-points of assessment by means of Student's t-test. The variables pH, density, total protein and chloride ion concentration in the AH were analyzed using the Kruskal-Wallis test. Binary variables defined in the terms of presence or absence (hyphema and synechia on physical examination, adhesions, fibrin, fibrosis and hemorrhage on histopathological assessment) were analyzed by means of bootstrapping for dichotomous data and the Wilcoxon test. P values below 0.05 ($p<0.05$) were considered to be statistically significant.

Results

At the neurophthalmological examination all observed parameters were kept normal in both groups throughout the experiment. Significant differences in IOP between TG and UG were not found at any time-point of evaluation. Nevertheless, the IOP increased at TP_1 in both groups. At TP_3 and TP_7, the IOP returned to the baseline values, while at TP_{15}, the IOP was below the baseline values at TP_0 and returned to the normal range at TP_{21} (Table 2).

Diffuse corneal opacity was found after surgery in both groups, without significant differences between them at any time-point of assessment. Intragroup comparisons of the various time-points showed greater degrees of opacity at TP_1, TP_3 and $TP_{7,}$ with statistical significance between TP_1 and TP_{21} in the UG (Graphic 1, Figure 1).

Aqueous flare occurred in both groups, without significant differences. In both groups, this parameter exhibited significant differences between TP_1 and TP_{15} and between TP_1 and TP_{21} (Graphic 1, Figure 1).

Fibrin formation was discrete and moderate in both groups, without significant differences between groups or at time-points of assessment (Graphic 1, Figure 1).

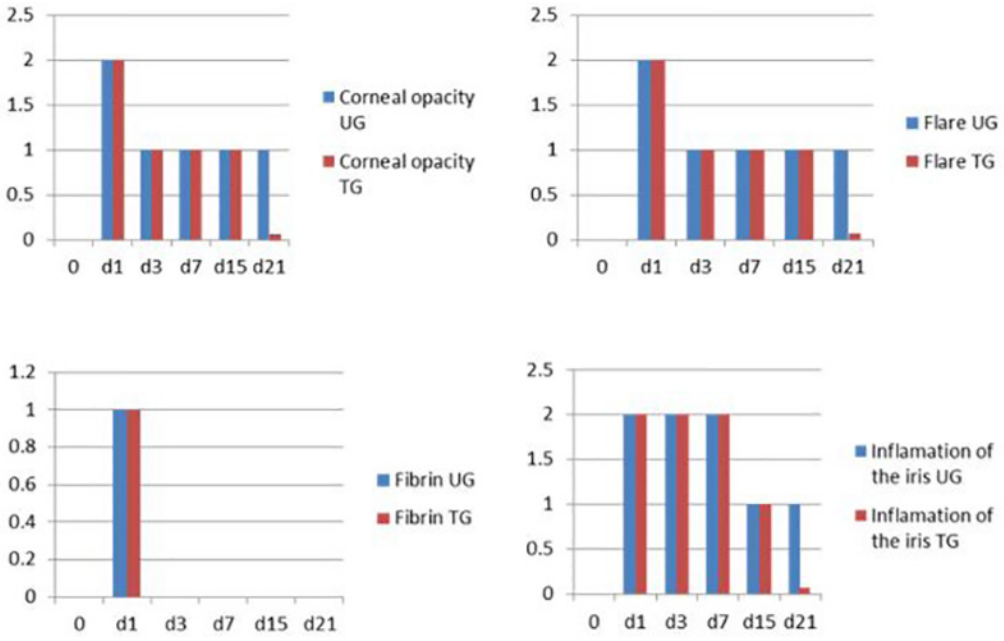
Iris inflammation, clinically manifested as edema and hyperemia, did not significantly differ between the two groups. In both groups, inflammation increased between TP_1 and TP_{21} and between TP_3 and TP_{21} (Graphic 1, Figure 1).

Hyphema was detected in one eye (8.33%) from an animal in the UG, related to a transoperative complication. Both groups exhibited occurrence of synechia from TP_1 to TP_{21}, without statistically significant differences.

Table 2. Means and standard errors for IOP of New Zealand rabbits subjected to phacoemulsification and trans operatively treated (TG) or not (UG) with intracameral TNK ($p<0.05$)

Time-point	IOP (mmHg)		p value		
	UG	TG	p*	p**	p***
TP_0	12.08 (2.26)	11.11 (1.65)	0.13		
TP_1	25.77 (13.10)^A	19.32 (12.79)^B	0.20	0.0017	0.0046
TP_3	11.10 (4.13)	10.49 (3.24)	0.64	0.335	0.59
TP_7	9.92 (2.66)^A	9.88 (3.61)	0.97	0.029	0.18
TP_{15}	7.99 (3.61)^A	8.05 (3.41)^B	0.95	0.004	0.0018
TP_{21}	9.27 (3.39)	9.69 (2.83)	0.77	0.0618	1.28

*p values between UG and TG.
**p values between TP_0 and other time-points for UG.
***p values between TP_0 and other time-points for TG.
Student's t-test at 5% significance.



Graphic 1 - Mean of corneal opacity, aqueous flare, anterior chamber fibrin and iris inflammation and representative images of eyes of New Zealand White male rabbits submitted to the phacoemulsification procedure and distributed in UG groups (untreated group) and TG (group treated with tenecteplase 0.1 ml to 50 µg intracameral intraoperatively) at different times of clinical evaluation.

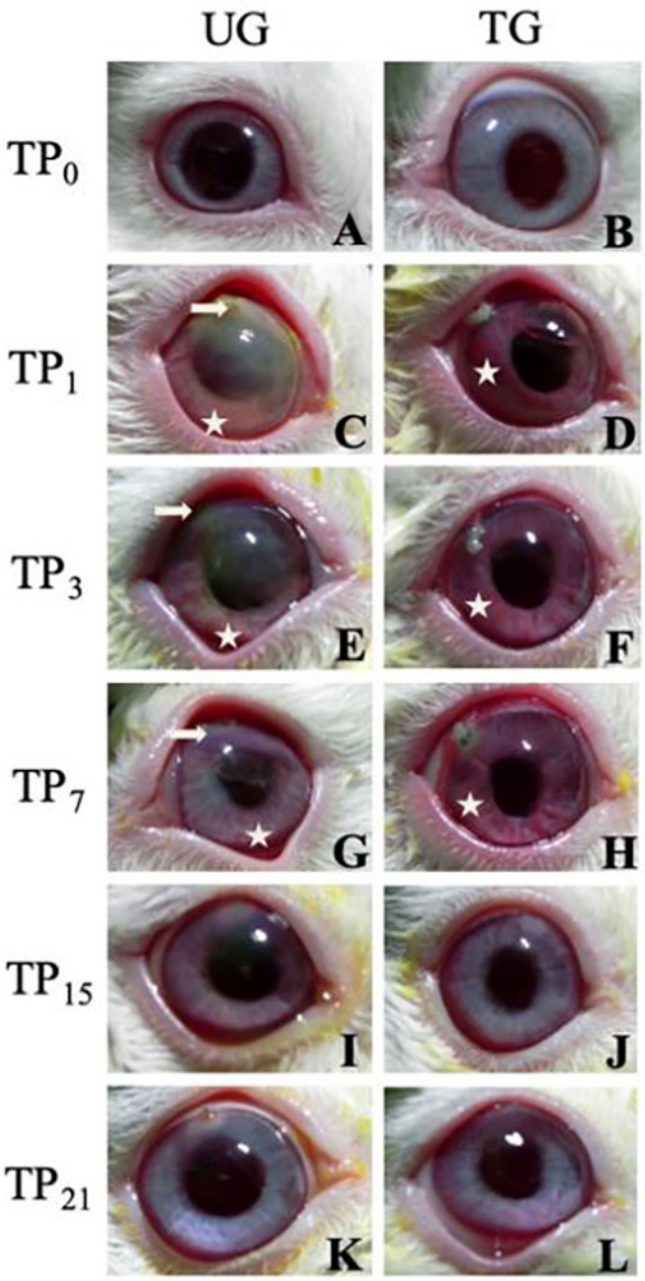


Figure 1. The images illustrate the moments of clinical evaluations (corneal opacities, anterior chamber evaluation, and iris evaluation) that were submitted to the animals. TP_0 - preoperative period; TP_1 - first postoperative day; TP_3 - third postoperative day; TP_7 - seventh postoperative day; TP_{15} - 15th postoperative day and TP_{21} - 21st postoperative day. The white arrows point to the area of corneal opacity and the stars indicate the area of inflammation of the iris identified by the change of coloration in relation to TP_0

Biochemical analysis of the aqueous humor

Table 3 describes the mean values of the AH pH, density and chloride ion (Cl^-) and total protein concentrations for both groups at TP_{21}.

The CG mean pH value (8.0 ± 0.22) did not exhibit a significant difference compared with the TG and UG. The mean density found in the CG, 1.008 ± 0.0004, significantly differed relative to the other two groups. The mean chloride ion concentrations (mEq/ml) did not significantly differ among the three groups. The groups also did not differ regarding the AH total protein concentration (mg/dl).

Table 3. Means and standard errors for aqueous humor pH, density and chloride ion and total protein concentrations among New Zealand rabbits at TP_{21}

Parameters	Results	CG	UG	TG	p
pH	Mean	8	8.1	8	0.558
	Standard error	(0.22)	(0.10)	(0.10)	
Density	Mean	1.008	1.012	1.011	0.0187
	Standard error	(0.0004)	(0.001)	(0.0019)	
Chloride (mEq/ml)	Mean	170.25	151.83	176.78	0.7844
	Standard error	12.33	9.89	19.15	
Proteins (mg/dl)	Mean	435	998.33	855	0.1955
	Standard error	(219.26)	(176.17)	(272.45)	

Kruskal-Wallis test at 5% significance.

Table 4 describes the histological findings relative to the presence of fibrin, fibrosis, hemorrhage and adhesions involving the ciliary body at TP_{21}.

While statistically significant differences were not found among the groups, the incidence rates of synechia, fibrin, fibrosis and adhesions were higher in UG.

Table 4. Frequency of histological changes in eyeballs of New Zealand White rabbits distributed per treated (TG) and untreated (UG) groups at TP_{21}

Histological changes	TG	UG
Adhesions	16.76%	33.40%
Fibrin	49.91%	83.11%
Fibrosis	24.80%	41.28%
Hemorrhage	66.51%	57.97%
Ciliary adhesions	18.30%	36.36%

Discussion

PHACO is the most widely used technique for treatment of cataracts as a function of its high rates of success and because it is less invasive than other techniques[1, 2]. These indices can be observed in the recovery of the operated animals, which underwent periodic evaluation without loss of vision, confirmed by the positive glare test since TP_1. However, despite its advantages, some complications may arise in the immediate postoperative period, such as corneal endothelial abnormalities, uveitis and fibrin formation in AC due to the handling of intraocular structures[3,4,20], which also occurred in the present study. It is noteworthy that the use of ultrasound was minimal in both groups, because of this it was not considered its impact on the postoperative inflammatory process.

In the present study, the use of TNK did not influence IOP in the postoperative period. Similar study[11], did not find that the use of TPA during PHACO in humans influences IOP. The peak of hypertension observed 24 hours after surgery was described as a common postoperative effect in rabbits. Such an occurrence was associated with BAB disruption, excessive motion of fluids during irrigation, manipulation of intraocular structures and even permanence of viscoelastic agents in the AC[7,8,20,21]. The IOP reductions observed at TP_{15} compared with TP_0 in both groups were likely a reflection of postoperative uveitis. Uvea inflammation is attended by a decrease in AH secretion by the ciliary body epithelium, resulting in ocular hypotension[22].

TPAs are trans operatively used in cases of congenital cataracts among humans[14] due to the high rates of fibrin formation in the AC. TNK is a third-generation TPA with high specificity for fibrin and high resistance to the action of the plasminogen activator inhibitor (PAI-1) enzyme secreted by platelets, thus more effectively decreasing the formation of fibrin clots however[23], this effect was not found in the animals from TG in the present study.

The toxicity of TNK was described in a rabbit-dose-dependent study (50-350μg)[15]. The authors observed that at the concentration of 50μg intravitreally was considered safe with no evidence of retinal toxicity in ophthalmoscopy, electroretinography and histology. At the dose of 150μg of TNK in ophthalmoscopy it was within normal range, however the histology showed mild retinal damage. Doses between 200-350μg presented evidence of toxicity in the electroretinography, ophthalmoscopy and histology. Other studies show that TNK at the dose of 50μg has low toxicity in the corneal endothelium and retina of rabbits and horse[10,16]. Therefore, in our study the corneal opacity detected in all of the analyzed eyes was attributed to corneal edema due to endothelial lesions caused by the energy generated during lens emulsification, fluid agitation in the AC and postoperative inflammation[20], regardless of the use or not of TNK[20]. Increased corneal opacity at TP_1, TP_3 and TP_7 coincided with the most important periods of iris inflammation, which points to uveitis as a factor contributing to the occurrence of corneal edema.

Aqueous flare was detected in the postoperative period in both groups. The aqueous flare is due to the presence of plasma proteins and cell components derived from postoperative uveitis and BAB disruption[8]. In the TG, starting at TP_{15}, a larger number of eyes did not exhibit aqueous flare, which might be a reflection of BAB stabilization and lower degrees of uveal inflammation[14,24]. Additional studies are needed to more accurately establish whether TNK may influence BAB and intraocular inflammation.

The fibrin formation is associated with tissue injury, migration of inflammatory cells and platelets and cytokine release. The fibrinolytic system (plasminogen-plasmin) is responsible for the dissolution of fibrin clots. It is activated when TPA converts plasminogen into plasmin, triggering fibrin degradation[25]. The studies investigated the use of intracameral TPA in the immediate postoperative period after PHACO in humans and found dissolution of the fibrin clots[11,14]. On these grounds, the expected outcome was a decreased presence of fibrin in the TG as a function of the fibrinolytic action of TPA. The lack of a difference in fibrin formation between the groups might be associated with severe postoperative inflammation and considerable stimulation of fibrin formation since inflammation and fibrinogenesis are triggered by similar activators in the presence of tissue injury[25]. A more effective postoperative protocol, including systemic and local anti-inflammatory agents, could have been applied to all of the rabbits undergoing surgery to better assess the action of TNK. Besides that this protocol could be extrapolated to other species of domestic animals. In addition, serial AH assessment based on the measurement of specific markers for fibrinolysis, such as fibrin degradation products (FDPs), could have contributed to the assessment of the action of intracameral TNK[24]. Other techniques could also have been used to achieve a better assessment of abnormalities in the cornea and the AH. Specular microscopy provides a detailed assessment of the density and morphology of the cornea and, thus, of damage in the corneal endothelium[26].

Iris inflammation occurred in both groups, coinciding with the higher frequencies of corneal opacity and aqueous flare. The appearance of signs of inflammation of the anterior segment is common after PHACO, possibly due to the manipulation of

instruments, contact of the phacoemulsifier probe tip with eye structures or iris prolapse[20,27].

Study recommended the transoperative use of TPA during PHACO in dogs to reduce the incidence of synechia[24]. However, in the present study, synechia occurred in both groups starting at TP_1 and remained until TP_{21}. A higher incidence of synechia was detected on the pupillary margin, where fibrin filaments were most often adhered, similar to the findings reported by Pena et al.[11] relative to the postoperative period following PHACO among humans. Synechia is the most frequent sequela of uncontrolled uveitis, and the use of anti-inflammatory drugs in the postoperative period has been recommended as a prophylactic measure[13].

Changes in AH composition have been attributed to BAB disruption in cases of tissue injury, intraocular surgery and chronic inflammation due to the extravasation of cells, platelets and inflammatory mediators into the AH[28,29]. In the present study, the AH pH did not differ among the groups, and its values agree with those reported by Pietrowskaa[30]. Changes in the AH pH are related to corneal edema, as a function of the occurrence of tissue hypoxia and the inefficiency of the endothelial pumps[31].

The AH density was higher in the TG and the UG than in the CG, likely due to PHACO-induced inflammation. Similarly, several studies found increased AH density in cases of intraocular inflammation, likely due to the presence of inflammatory cells and proteins. Total protein concentrations were higher in the TG and the UG than in the CG[7,29]. In addition, considered the total protein concentration as an indicator of intraocular inflammation. Mauri et al.[32] related the presence of aqueous flare and iris hyperemia and edema with an increase in the total protein concentration in the AH, as observed in the present study.

Chloride ion concentrations did not exhibit statistically significant differences among the groups. Mauri et al.[32] described the participation of chloride ions in the production of AH and in carbonic anhydrase activation and, therefore, in the control of IOP. In the present study, no correlation was found between IOP and chloride concentrations in AH.

The fibrosis and adherence found in the ciliary processes confirmed the occurrence of postoperative uveitis. These abnormalities were correlated with the presence of protein exudate in the AC due to fibrin extravasation and cell infiltrates in response to PHACO[33,34] and tissue repair. The incidence of fibrin presence and fibrosis was lower in the TG, as also described by Escalina et al.[24] due to the fibrinolytic action of TNK, which resulted in reduction of the fibrin deposits. However, the incidence of hemorrhage was higher in this group, which might have been due to tissue injury following excessive manipulation of the intraocular structures[20]. In addition, TNK might cause superficial bleeding at the site of application due to the lysis of the fibrin in the clot during the first hours following application of the drug. However, several studies have shown that use of TPA for treatment of eye problems was associated with low rates of hemorrhagic complications[11,12,13,14,19,24].

The AH and eyeballs were assessed at a single time-point (TP_{21}). Serial assessment during

the postoperative period might provide more information on AH composition and histological changes, resulting in a more accurate representation of the physicochemical phenomena and cell responses in the AC of rabbits subjected to PHACO.

Further studies with intracameral TNK in the postoperative period of PHACO at different concentrations are recommended to verify its effectiveness in controlling the fibrin deposition in rabbits.

Conclusions

Transoperative use of TNK at a concentration of 50 µg / 0,1ml for PHACO in rabbits exerted no influence on the IOP, corneal opacity, signs of uveitis or fibrin formation in the AC, on the physicochemical parameters of AH or on the eyeball histopathology.

Acknowledgments

The first author is grateful to the National Council for Scientific and Technological Development (CNPq) for the research scholarship awards that supported this project. Special thanks to all postgraduate and undergraduate students and professors from the Veterinary Hospital and Multidisciplinary Laboratory of Clinical Pathology of the School of Veterinary and Animal Science, Laboratory of Pharmacology and Cell Toxicology of the Faculty of Pharmacy from the Federal University of Goiás who were involved in this project.

The authors declare that they have no conflicts of interest.

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