Subconjunctival and topical application of recombinant tissue plasminogen activator in rabbits

Uso tópico e subconjuntival de ativador de plasminogênio tecidual recombinante em coelhos

JOSÉ RICARDO DE ABREU REGGI1, RICHARD YUDI HIDA1,2, MILTON MASSATO HIDA3, MARIA CRISTINA NISHIWAKI-DANTAS1, HISASHI SUZUKI2

INTRODUCTION

The development of exuberant fibrinous exudation or inflammation is a rare but serious complication after ocular surgery. It can cause numerous sequelae including membranes, anterior or posterior synechiae, secondary glaucoma, and pupillary block. Previous studies showed that recombinant tissue plasminogen activator (r-TPA) is effective and safe for improving the clinical course of fibrin-related complications in cases of traumatic hyphema, glaucoma surgery, subretinal hemorrhage, and endophthalmitis.

Intracameral r-TPA has been shown to be effective for fibrin degradation. However, the effectiveness of topical, intravitreal, and subconjunctival r-TPA remains controversial. Most studies regarding absorption or anterior chamber dosing of r-TPA were non-quantitative. To the best of our knowledge, no study has compared intracameral, subconjunctival, and topical application of r-TPA using the same quantitative methods.

Therefore, the purpose of this study was to quantify fibrin degradation products (FDP) after topical and subconjunctival administration of r-TPA in rabbits.

METHODS

This prospective double-blind experimental study was performed in the Setor de Técnica Cirúrgica da Santa Casa de São Paulo under veterinary supervision. It was approved by the Animal Ethical Committee and Institutional Board of the Hospital das Clínicas and the Faculdade de Medicina da Universidade de São Paulo. All procedures followed proper legislation for the protection of animals (EU Directive 2010/63/EU) and adhered to the Association of Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research.

Twenty-five New Zealand male rabbits weighing 3.5 to 4.0 kg were enrolled in this study. All rabbits underwent an ocular examination and slit lamp documentation (Nikon, FS3 slit lamp, Japan). All rabbits with ocular abnormalities were excluded from the study.

Anesthesia was induced in each rabbit using intramuscular injection of a mixture of 0.3 mL/kg tiletamine hydrochloride and zolazepam (Zoletil 50®, Laboratoire Virbac, France). General anesthesia was achieved with an intramuscular injection of 0.4 mL/kg fentanyl and droperidol (Innovar-Vet®, MTC Pharmaceuticals, Ontario, Canada).

Submitted for publication: June 30, 2014
Accepted for publication: November 10, 2014
1 Department of Ophthalmology, Santa Casa de São Paulo, São Paulo, SP, Brazil.
2 Department of Ophthalmology, University of São Paulo (USP), São Paulo, SP, Brazil.
3 Department of Ophthalmology, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, SP, Brazil.

Funding: No specific financial support was available for this study.
Disclosure of potential conflicts of interest: None of the authors have any potential conflicts of interest to disclose.
Corresponding author: Richard Yudi Hida. Rua Afonso de Freitas, 488/61 - São Paulo, SP - 04006-052 - Brazil. E-mail: ryhida@gmail.com

http://dx.doi.org/10.5935/0004-2749.20150003

ABSTRACT
Purpose: To quantify fibrin degradation products after topical and subconjunctival administration of recombinant tissue plasminogen activator in rabbits.

METHODS: Fibrin formation was induced in the anterior chamber in 25 rabbits. Subsequently, five rabbits received an injection of r-TPA (positive control) in the anterior chamber, another 10 received a subconjunctival injection of r-TPA, and the remaining 10 received instillations of topical r-TPA. Afterwards, samples of aqueous humor were collected and semi-quantitative analysis of fibrin degradation products (FDP) was performed.

RESULTS: No statistical differences were noted between the treatment and control groups at any time point. Fibrin degradation products semi-quantification showed statistical improvement in the control group and the subconjunctival group.

Conclusion: Fibrin degradation products were observed in the anterior chamber after subconjunctival administration of r-TPA. However, it was probably not sufficient to cause fibrin degradation. Topical r-TPA did not effectively absorb anterior chamber fibrin.

Keywords: Tissue plasminogen activator; Anterior chamber; Hyphema; Inflammation; Postoperative complications; Animals; Rabbits

RESUMO
Objetivo: Quantificar produtos de degradação de fibrina (PDF) após uso tópico e subconjuntival de ativador de plasminogênio tecidual recombinante (r-TPA) em coelhos.

Métodos: Formação de fibrina foi induzida na câmara anterior em 25 coelhos. Cinco coelhos foram submetidos a injeção intracameral de r-TPA (controle positivo). Dez coelhos foram submetidos a injeção subconjuntival de r-TPA e dez coelhos foram submetidos a instilação tópica de r-TPA. Amostras de humor aquoso foram coletadas e uma análise quantitativa dos produtos de degradação de fibrina foi realizada.

Resultados: Não foi observado diferença estatisticamente significativa na degradação de fibrina em nenhum dos momentos estudados quando comparados com o controle. Porém foi observado diferença estatisticamente significante na quantificação dos produtos de degradação de fibrina no grupo controle e no grupo subconjuntival.

Conclusão: Produtos de degradação de fibrina foi observado nas amostras do grupo subconjuntival, porém, provavelmente não foi suficiente para degradar a fibrina presente. r-TPA tópico não foi efetivo em absorver fibrina na câmara anterior.

Descritores: Ativador de plasminogênio tecidual; Câmara anterior; Hifema; Inflamação; Complicações pós-operatórias; Animais; Coelhos

Discritores: Ativador de plasminogênio tecidual; Câmera anterior; Hifema; Inflamação; Complicações pós-operatórias; Animais; Coelhos

INTRODUCTION

The development of exuberant fibrinous exudation or inflammation is a rare but serious complication after ocular surgery. It can cause numerous sequelae including membranes, anterior or posterior synechiae, secondary glaucoma, and pupillary block. Previous studies showed that recombinant tissue plasminogen activator (r-TPA) is effective and safe for improving the clinical course of fibrin-related complications in cases of traumatic hyphema, glaucoma surgery, subretinal hemorrhage, and endophthalmitis.

Intracameral r-TPA has been shown to be effective for fibrin degradation. However, the effectiveness of topical, intravitreal, and subconjunctival r-TPA remains controversial. Most studies regarding absorption or anterior chamber dosing of r-TPA were non-quantitative. To the best of our knowledge, no study has compared intracameral, subconjunctival, and topical application of r-TPA using the same quantitative methods.

Therefore, the purpose of this study was to quantify fibrin degradation products (FDP) after topical and subconjunctival administration of r-TPA in rabbits.
After appropriate anesthesia was attained, 5.0 mL of blood was collected from the ear vein and centrifuged to isolate the plasma. Each rabbit received topical 0.5% procaine chloride (Anestalcon®, Alcon, São Paulo, Brazil), and then a lid speculum was used for paracentesis in the superior portion of the cornea with a 25-gauge needle. Keeping the needle in the anterior chamber, 0.1 mL of aqueous humor was aspirated and 0.1 mL of plasma citrate was injected.

After 24 h, all rabbits were sedated and photographed under a slit lamp. All eyes with fibrin in the anterior chamber were graded according to the classification described by Lim et al.14,15 (grade 0: no visible fibrin; grade 1: few visible fibrin filaments and clear details of the iris; grade 2: presence of a fibrin clot and blurry details of the iris; grade 3: presence of a fibrin clot, no visible details of the iris). All eyes with fibrin levels of grade 1 and 2 of fibrin in the anterior chamber were excluded from the study. Sampling calculations were based on a previous pilot study and statistical analysis was performed using the WINPEPI program with the COMPARE2 module (Version 2.68) to avoid sampling error. The sample size was increased in 20% due to possible loss to follow-up.

Rabbits were divided into three study groups: group 1 (n=5; positive control) received intracameral injection of r-TPA; Group 2 (n=10) received subconjunctival injection of r-TPA; Group 3 (n=10) received topical r-TPA.

One eye from each rabbit in group 1 was randomly chosen (positive control) to receive an injection of 0.1 mL of 0.25 µg/mL r-TPA (TPA-Alteplase®, Ophthalmos, São Paulo, Brazil) in the anterior chamber after instillation of topical 0.5% procaine chloride (Anestalcon®, Alcon, São Paulo, Brazil). The other eye from the same rabbit was injected with 0.1 mL balanced salt solution using the same technique.

One eye from each rabbit in group 2 was randomly chosen to receive a subconjunctival injection of 0.1 mL of 0.25 µg/mL r-TPA after instillation of topical 0.5% procaine chloride. The other eye was injected with 0.1 mL balanced salt solution using the same technique.

One eye from each rabbit in group 3 was randomly chosen to receive one drop of topical r-TPA at 1 mg/mL, 9 times at intervals of every 5 min after instillation of topical 0.5% procaine chloride. The other eye received one drop of 0.1 mg/mL balanced salt solution in the same regimen.

Each rabbit was examined, classified, and documented under sedation using a slit lamp (Nikon, model FS3, Japan) at the moment of the procedure (M0), and 30 min (M1), 60 min (M2), 7 h (M3) and 24 h (M4) after the procedure.

After 24 h, each rabbit was euthanized with intramuscular 1 mg/kg acepromazine and intravenous 30 mg/kg thiopental sodium. All eyes underwent paracentesis and aspiration of samples of aqueous humor using a 25-gauge needle. Samples were immediately transported to our laboratory for qualitative and semi-quantitative analysis of FDP and fibrinogen using the macro-latex slide agglutination test (FDPlasma®, Diagnostica Stago Inc, France) according to the manufacturer’s instructions. This test involves multiple microlatex particles coated with mouse monoclonal anti-human FDP antibodies13. Presence of FDPs causes agglutination of the latex particles as the FDPs bind to the antibodies. Then these agglutinated particles are detected visually. The detection limit of this test is 2.5 µg/mL.

Qualitatively, the agglutination pattern was interpreted as negative when transparent and positive when blurred. Quantitatively, a positive agglutination pattern was interpreted as recommended in the manufacturer’s instructions.

All results were analyzed statistically with the Wilcoxon test (p<0.05).

All animal care and procedures were performed under the supervision of a single veterinarian to avoid bias and factors that could influence the hemodynamics and neuroendocrinological conditions of the animals (anesthesia technique, stress, food, etc.). All animals were kept in individual cages and given water and food ad libitum. Light, temperature, and humidity were controlled and monitored.

**RESULTS**

Table 1 shows the averages and standard deviations of anterior chamber fibrin grading following Lim et al.14 after application of r-TPA.

In group 1 (intracameral administration), no statistically significant difference was noted at M1 compared to the control group (p=0.06). However, anterior chamber fibrin grading showed statistically significant improvement at M2, M3, and M4 compared to the control group (p=0.0079).

In group 2 (subconjunctival administration), no statistically significant difference was noted at any time point (M1, M2, M3 and M4) compared to the control group (p>0.05).

In group 3 (topical administration), no statistically significant difference was noted at any time point (M1, M2, M3 and M4) compared to the control group (p>0.05).

Table 2 shows the results of the semi-quantification of the FDPs following the different applications of r-TPA compared to the control group.

FDP semi-quantification showed statistically significant improvement in groups 1 (p=0.0079) and 2 (p=0.0052), but no statistically significant difference was observed in group 3 (p=0.48).

**DISCUSSION**

Fibrinous exudation is a serious complication of intraocular surgery and endophthalmitis. These complications can preclude fundus examination and interfere with the performance of a second intervention such as vitrectomy. These fibrinous membranes are usually difficult to manage with conventional steroid therapy. Typically, intraocular fibrin can be surgically removed, treated with an argon laser, or

<table>
<thead>
<tr>
<th>Examination time points</th>
<th>A ± SD</th>
<th>p</th>
<th></th>
<th>A ± SD</th>
<th>p</th>
<th></th>
<th>A ± SD</th>
<th>p</th>
<th></th>
<th>A ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
<td>M2</td>
<td>M3</td>
<td>M4</td>
<td></td>
<td></td>
<td>M1</td>
<td>M2</td>
<td>M3</td>
<td>M4</td>
<td></td>
</tr>
<tr>
<td>Group intracameral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.00 ± 0.00</td>
<td>0.06</td>
<td></td>
<td>3.00 ± 0.00</td>
<td>0.00</td>
<td></td>
<td>2.80 ± 0.45</td>
<td>0.00</td>
<td></td>
<td>2.00 ± 0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>(0.25 µg/mL) r-TPA</td>
<td>1.80 ± 0.84</td>
<td>0.97</td>
<td></td>
<td>1.60 ± 0.55</td>
<td>0.97</td>
<td></td>
<td>1.00 ± 0.00</td>
<td>0.00</td>
<td></td>
<td>0.20 ± 0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Group subconjunctival</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.90 ± 0.32</td>
<td>0.00</td>
<td></td>
<td>2.90 ± 0.32</td>
<td>0.00</td>
<td></td>
<td>2.70 ± 0.48</td>
<td>0.00</td>
<td></td>
<td>2.10 ± 0.32</td>
<td>0.00</td>
</tr>
<tr>
<td>(0.25 µg/mL) r-TPA</td>
<td>3.00 ± 0.00</td>
<td>0.00</td>
<td></td>
<td>3.00 ± 0.00</td>
<td>0.00</td>
<td></td>
<td>2.70 ± 0.48</td>
<td>0.00</td>
<td></td>
<td>2.00 ± 0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>Group topical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.00 ± 0.00</td>
<td>0.00</td>
<td></td>
<td>3.00 ± 0.00</td>
<td>0.00</td>
<td></td>
<td>2.50 ± 0.53</td>
<td>0.00</td>
<td></td>
<td>2.20 ± 0.42</td>
<td>0.00</td>
</tr>
<tr>
<td>(1 mg/mL) r-TPA</td>
<td>3.00 ± 0.00</td>
<td>0.00</td>
<td></td>
<td>3.00 ± 0.00</td>
<td>0.00</td>
<td></td>
<td>2.60 ± 0.52</td>
<td>0.00</td>
<td></td>
<td>2.30 ± 0.48</td>
<td>0.00</td>
</tr>
</tbody>
</table>

r-TPA= recombinant tissue plasminogen activator; A= average; SD= standard deviation; M1= anterior chamber fibrin grading scale 30 min after r-TPA application; M2= anterior chamber fibrin grading scale 60 min after r-TPA application; M3= anterior chamber fibrin grading scale 7 h after r-TPA application; M4= anterior chamber fibrin grading scale 24 h after r-TPA application.
Subconjunctival and topical application of recombinant tissue plasminogen activator in rabbits

Table 2. Distribution of average and mean deviation of the dilution(2,29) of fibrin in anterior chamber with subconjunctival and topical injection of r-TPA in rabbits. p<0.0005.

<table>
<thead>
<tr>
<th>Level of FDP (µg/mL)</th>
<th>A ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group intracameral</td>
<td>Control</td>
<td>2.50 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>r-TPA</td>
<td>12.00 ± 4.47</td>
</tr>
<tr>
<td>Group subconjunctival</td>
<td>Control</td>
<td>2.50 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>r-TPA</td>
<td>4.50 ± 1.05</td>
</tr>
<tr>
<td>Group topical</td>
<td>Control</td>
<td>2.50 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>r-TPA</td>
<td>2.75 ± 0.79</td>
</tr>
</tbody>
</table>

r-TPA = recombinant tissue plasminogen activator; FDP = fibrinogen degradation products; A = average; SD = standard deviation; Wilcoxon test (**P<0.0005).

Dr. Abreu Reggi and Hida contributed equally to this work and request acknowledgement as co-first authors.

ACKNOWLEDGMENT

REFERENCES