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Tissue plasminogen activator reduces the elevated intraocular pressure induced by prednisolone in sheep

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ABSTRACT

We have previously shown that tissue plasminogen activator (tPA) injected in the vitreous of sheep, reduced or prevented the elevation of the intraocular pressure (IOP) normally produced by the instillation of 1% prednisolone. We now report the effect of tPA when injected into the anterior chamber (AC) in amounts of 0.01, 0.001 and 0.0001 μg diluted in a volume of 50 μL. Lyophilized tPA, obtained as Actilyse® 50 mg from Boehringer Ingelheim containing arginine was utilized. The Actilyse was diluted in balanced salt solution to obtain the desired amount of tPA in 50 μL. An identical solution containing only arginine was prepared to inject into the contralateral eye as a control. Six sheep of the Corriedale breed were selected. At the beginning of the study all eyes received instillation of 1% prednisolone 3 times/day for 10 days to elevate their IOP from 10 mm Hg to about 23 mm Hg. Then, 0.0001 μg was injected into one of the eyes and its effect was followed for up to 55:00 h while the instillation of prednisolone continued in both eyes. The same protocol was implemented for the 0.001 and 0.01 μg amounts after extended washout and IOP was over 22 mm Hg. The injection of 0.0001 μg into the AC had no effect on an IOP of 23.0 mm Hg at 6:00 and 30:00 h after injection. 0.001 μg of tPA reduced IOP from 23.1 to 18.6 mm Hg at 6:00 h but IOP recovered to 22.3 mm Hg at 30:00 h. Injection of 0.01 μg produced a marked and prolonged reduction of IOP. From a baseline of 23.0, IOP was reduced to 14.0, 14.7, 21.2, and 20.9 mm Hg at 5.0, 23.0, 27.0 and 45.5 h, respectively. The 0.423 μg of arginine, which is associated with 0.01 μg tPA, was injected alone and had no effect. Recombinant human tPA injected in the AC is effective in reversing steroid-induced IOP elevation in sheep. The reduction of IOP elevation may be the result of an effect on extra-cellular matrix turnover in the TM. These findings suggest that tPA may by useful as a therapeutic agent in steroid-induced glaucomas.

We have previously shown that the tissue plasminogen activator (tPA) injected into the vitreous of sheep either reduced the prednisolone-elevated intraocular pressure (IOP) or prevented the IOP increase when injected simultaneously with the cortico-steroid (Gerometta et al., 2013). The effect was observed with injections of 100 μg to 1 mg and lasted for about 9 days. We reasoned that tPA was released from the vitreous and reached the trabecular meshwork (TM) were degraded the accumulated collagen. Thus, we decided to investigate if a single injection of tPA into the anterior chamber (AC) also reduced the IOP, and in what amount and time sequence. This would be important as an eventual treatment in which drops of tPA are instilled in the conjunctival sac.

Tissue plasminogen activator (tPA) is a serine protease that catalyzes the conversion of the inactive plasminogen into plasmin, the major degradative enzyme of blood clots via the proteolytic degradation of fibrin (Lijnen and Collen, 1995). This cascade can also lead to the activation of other pro-enzymes, including members of the matrix-metalloproteinase (MMP) family of enzymes, into their active forms (Matrisian, 1990; Murphy et al., 1992).

The MMPs directly degrade extracellular matrix (ECM) components, and these enzymes play a key role in the turnover and maintenance of the ECM of the TM, a process affecting outflow facility (Bradley et al., 1998; Keller et al., 2009).

tPA is expressed and secreted by various organ systems including the TM (Park et al., 1987; Shuman et al., 1988), and is found in the aqueous humor (Tripathi et al., 1988), suggesting that tPA could have an important role in regulating ECM composition in the TM. Glucocorticosteroids increase the accumulation of ECM

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components in the TM and decrease outflow facility, and have been shown to elicit reductions in tPA activity in TM organ and cell cultures (Seftor et al., 1994), suggesting a link between these phenomena.

In past work, we demonstrated the effectiveness of glucocorticosteroids to induce ocular hypertension in Corriedale sheep (Gerometta et al., 2009). The IOP of these animals increased over 2-fold within 1–2 weeks of topically applying either 0.5 or 1.0% prednisolone acetate, three times daily. This IOP elevation occurred with a 100% incidence in the corticosteroid-treated eyes. Using prednisolone, the IOP of sheep will remain elevated as long as the instillation regimen is maintained.

All animal experiments were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) guidelines. A total of 6 healthy female sheep (Corriedale breed) between 16 and 24 months of age, and weighing 35–40 kg, were selected from a local ranch in Corrientes, Argentina for this study. The eyes and general health of the animals were considered normal by an ophthalmologist and a veterinarian, respectively. Sheep were tagged for individual identification on their ear lobes and herded from pasture whenever it was necessary to 1) topically instill prednisolone, 2) inject either tPA or vehicle with arginine intracamerally, or 3) measure IOP by applanation tonometry. For each of these procedures, the sheep were guided into a funnel corral ending in a loose-fitting yoke. This arrangement allowed movement and holding of the head by one person while another either instilled the prednisolone, injected tPA into de AC under local anesthesia, or measured IOP with a hand-held Perkins tonometer calibrated for the sheep eye (Gerometta et al., 2009). Between all procedures, the animals were free to pasture.

1. Prednisolone instillation

All sheep eyes received two drops of 1.0% prednisolone acetate ophthalmic suspension USP (manufactured by Alcon Laboratories, Inc. Fort Worth, Texas for Sandoz Inc. Princeton, NJ), 3-times daily (at 7 AM, 2 PM, and 7 PM) for the duration of the experiments. Such treatment induces a persistent ocular hypertension in sheep provided the instillation protocol is uninterrupted (Gerometta et al., 2009).

Lyophilized tPA was procured as Actilyse® from Boehringer Ingelheim SA (Buenos Aires, Argentina). 5 mg of Actilise containing 4.8856 of arginine and 0.1155 mg of tPA was dissolved in 577.4 mL of sterile balanced saline solution (BSS). 50 μL of this solution containing 0.01 μg of tPA was injected into the AC of the 6 sheep under local anesthesia (2 drops of topical 0.5% proparacaine, Alcon Laboratorios Argentina S.A.). Lower amounts of tPA (0.0001 and 0.001 μg) were prepared by appropriate dilution and injected into the AC. An amount of arginine, equal to that present in the 3 tPA amounts used, was injected into the contralateral eye.

As previously indicated, bilateral topical treatment with prednisolone for 10 days doubled IOP on both eyes of the sheep. Subsequent intracameral injection of tPA had a dose-dependent effect. 0.0001 μg had no effect on IOP. 0.001 μg had a small decreasing effect on IOP in some eyes 5 h after injection that lasted 23 h.

0.01 μg reduced IOP in all eyes from a mean of 23.0 to 14.0 mm Hg 5 h after injection. The reduction lasted at least 24 h and 48 h later a small effect persisted. The fellow eye was injected with the same amount of arginine contained in the Actilyse. All eyes remained normal without sign of congestion or inflammation. These results are summarized in Fig. 1 and Table 1.

The fibrinolytic system controls clotting of blood and subsequent dissolution of the thrombus. tPA activate plasminogen which then becomes plasmin that degrades fibrin (Lijnen and Collen, 1995). Many publications describe the use of human recombinant tPA either intracameraly for the acute management of excessive fibrin in the anterior segment of the eye (Wedrich et al., 1997; Wu and Wang, 2009; Erol et al., 2003; Ozveren and Eltutar, 2004). In those publications IOP reductions are mentioned but have been attributed to the dissolution of the fibrin clot in the anterior chamber. To date no experiments had been reported that use the fibrinolytic system in steroid-induced or open angle glaucoma. tPA has been used by intracameral injection at a dosage of 10–25 μg as a fibrinolitic in endophthalmitis and hyphema respectively (Wu and Wang, 2009; Kim et al., 1998). We found that amounts as low as 0.01 μg reduces IOP in only 4 h and the effect persists for at least 25 h. Possibly because of the low tPA/arginine dose that was

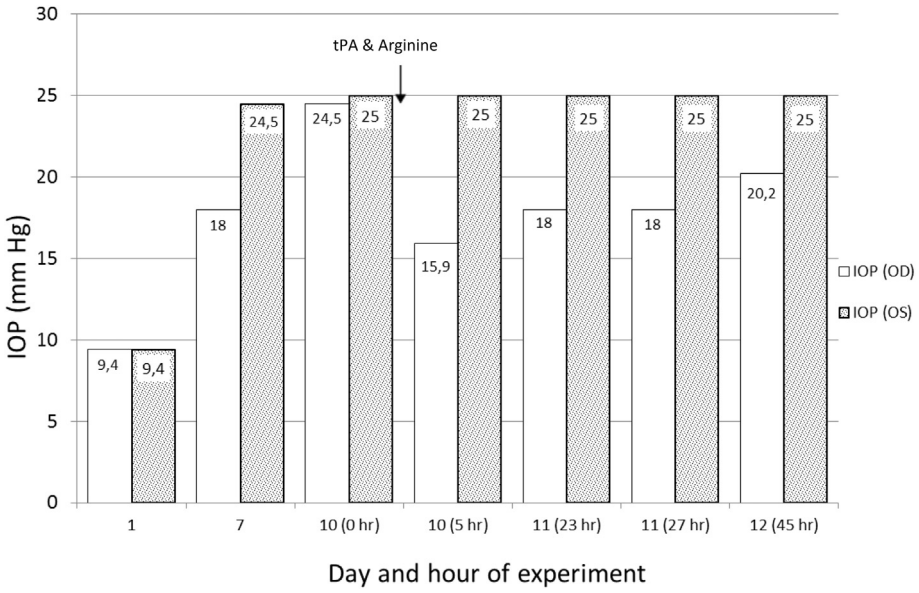

Fig. 1. Representative experiment of the effect of 0.001 μg of tPA when injected in the AC of the right eye. The AC of the left eye received arginine. There was a drastic reduction of the IOP of the right eye, which slowly recovered to the pre-tPA values despite the continued treatment with prednisolone. There was no effect of arginine on the IOP of the left eye.

Table 1
Progression of intraocular pressure of the tPA treated eye. (From Day 1 when prednisolone instillation started followed by Intracameral injection of tPA on Day 10).

	Prednisolone to both eyes ↓		tPA to one eye Arginine to contralateral ↓				
SHEEP #							
a-13	9.4	18	24.5	15.9	18.0	18.0	20.2
b-13	11.6	15.9	22.3	13.7	15.9	20.2	22.3
c-13	9.4	15.9	22.3	13.7	15.9	20.2	20.2
1-14	11.6	15.9	22.3	13.7	11.6	22.3	22.3
2-14	9.4	15.9	22.3	13.7	13.7	24.5	20.2
3-14	9.4	18.0	24.5	13.7	13.7	22.3	20.2
Mean	10.08	16.57	23.01	14.04*	14.66*	21.15	20.88
SD	1.14	1.08	1.14	0.90	2.25	2.26	1.08
SE	0.51	0.49	0.51	0.40	1.01	1.01	0.49
Day	1	7	10	10	11	11	12
Hour			0.0	5.0	23.0	27.0	45.5

Values are expressed in mmHg. *14.04 & 14.66 significantly smaller than 23.01; P<0.001 as paired data.
Raw data of the tPA treated eye has a grey background.
The contralateral eye received arginine, which did not reduce IOP.

injected in a volume of 50 μL into the AC, there were no signs of inflammation or toxicity. All eyes remained normal 2 weeks after completing the experiments. Although tPA has an effect on many enzymes, we posit that the reduction in IOP is due to its effect (direct or indirect) on the extracellular matrix of the TM. We realize that results of work on animal models may no necessarily indicate that similar mechanisms are operative in humans. Thus, they need to be confirmed with appropriate trials. Nevertheless, these findings could hold implications for the treatment of steroid-induced glaucoma and potentially in other open angle glaucoma.

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