Intravitreal Tenecteplase (Metalyse) for Acute Management of Retinal Vein Occlusions

Ian L. McAllister,1 Sarojini Vijayasekaran,1 and Dao-Yi Yu1,2

1Lions Eye Institute, Centre for Ophthalmology and Visual Science, University of Western Australia, Perth, Australia
2The Australian Research Council (ARC) Centre of Excellence in Vision Science, University of Western Australia, Perth, Australia

Correspondence: Ian L. McAllister, Lions Eye Institute, Centre for Ophthalmology and Visual Science, University of Western Australia, Nedlands, WA 6009, Australia; ianmcallister@lei.org.au.
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PURPOSE. To determine the ability of an intravitreal injection of tenecteplase (TNK) to penetrate an intraretinal venous thrombus and its effectiveness in thrombolysis in a porcine model of branch retinal vein occlusion (BRVO).

METHODS. Six pigs (group 1) were anesthetized, and a BRVO was induced photothermographically in the left eye; immediately afterward, fluorescence-conjugated TNK (100 µg) was injected into both eyes, with enucleation at 24 hours. Retinal penetration was assessed on frozen sections by epifluorescence microscopy. A further six pigs (group 2) were anesthetized; BRVO was induced in both eyes, and TNK was injected into the vitreous in the left eye. Both eyes were harvested a week later. The area of the lasered site and an area away from the burn were dissected and processed in epoxy resin and stained for light or transmission electron microscopy. The percentage blockage, clot volume, cytostructure, and extent of thrombolysis by TNK were assessed.

RESULTS. TNK penetrated the veins in both eyes of group 1 pigs, with more intense staining in the eyes with the occlusion. In group 2 eyes, thrombolysis was significant in the eyes injected with TNK (P = 0.03); blockage was seen in all six untreated eyes and one treated eye. Clot volume was significantly higher in untreated eyes (P = 0.028). Percentage blockage varied from 8.5% to 83.9%. Damage by TNK to the neural retina was not seen. There was no significant difference in cytostructure between treated and untreated eyes (P = 0.357).

CONCLUSIONS. TNK was able to penetrate the retinal veins with and without an occlusion and effect lysis of BRVO, and did not cause damage to the retinal tissue. Intravitreal TNK may be useful as an acute treatment for RVOs of recent onset.

Keywords: TNK, BRVO, vitreoretinal surgery

Retinal vein occlusions (RVO) remain a common cause of unilateral vision loss worldwide. They are the second most common cause of retinal vascular blindness after diabetic retinopathy, affecting approximately 16 million people worldwide.1–3 Approximately 1% of individuals younger than 60 years are affected, with the prevalence increasing to 5% in those over 80 years.2

Retinal vein occlusions may affect either a branch vein or the central retinal vein. Branch retinal vein occlusion (BRVO) is more common and accounts for 80% of all vein occlusions.2,4 Without treatment, it can lead to a sustained loss of vision, with a reported final mean visual acuity of 20/70 and 23% of patients having a visual acuity ≤ 20/200.5,6

The vision loss in acute BRVO is predominantly due to macular edema.7 Macular edema and retinal neovascularization are sequelae of the obstruction to venous outflow that occurs in this condition and have been the focus of most of the therapies that have been trialled for this condition to date.

Treatments that potentially directly intervene in the causative pathogenesis have been attempted, but as of yet none have progressed to the stage at which a randomized controlled trial has been performed. BRVOS always occur at an anatomically susceptible spot where an artery and vein cross and the common adventitial sheath binds these together. It is thought that the thickened and rigid arteriosclerotic arterial wall compresses the vein, resulting in turbulence in blood flow and endothelial damage leading to the formation of a local thrombus.8–10

The evidence that a thrombus is the cause of the obstruction to the venous outflow in BRVO is much stronger than in central retinal vein occlusion (CRVO), where both the cause and site of the venous obstruction still remain controversial.11–13 The largest histopathological study of BRVOs showed evidence of a fresh or recanalized thrombus within the retinal vein at the site of the obstruction in all nine cases examined.9 This finding has led to attempts to both relieve the localized obstruction by releasing the pressure on the retinal vein from the adjacent artery, via incision of the common adventitial sheath, and to directly lyse the intravascular thrombus with thrombolytic agents.

Vitrectomy surgery with decompression of the arteriovenous crossing has been attempted, with some reports suggesting a benefit and others finding no difference from vitrectomy alone.14–20 Direct attempts to lyse the presumed thrombus causing the obstruction to venous outflow have also been attempted in both CRVO and BRVO.21–26 These have included injections of tissue plasminogen activator (tPA) both into the vitreous and also directly into a branch vein using a microinjector system. The results from these studies have been
inconclusive, and intraocular thrombolytics for RVOs are not widely used.

Part of the problem with attempting to lyse the intravascular thrombus in RVOs has been with the agent chosen. Tissue plasminogen activator does not penetrate the retina well and also requires a prolonged clot contact time to be effective.\textsuperscript{27,28}

We have previously investigated the third-generation thrombolytic tenecteplase (TNK) as a treatment for submacular hemorrhage.\textsuperscript{29-31} TNK offers significant benefits for intraocular use compared to tPA in that it is less toxic, requires a much shorter clot contact time to be effective, and has been shown to freely penetrate the retina.

In this study we report results on the ability of an intravitreal injection of TNK to penetrate an intraretinal venous thrombus and also to effect a thrombolysis in a porcine model of BRVO.

**MATERIALS AND METHODS**

All animal procedures were approved by the Animal Ethics Committee of the University of Western Australia and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the policies in the Guide to the Care and Use of Laboratory Animals issued by the National Institutes of Health. A total of 12 pigs were used, 6 to ascertain if TNK can penetrate retinal veins with and without a thrombus, and 6 to determine the thrombolytic properties of and retinal damage by TNK in RVO.

**Surgery and Tissue Processing**

Six pigs (group 1) (aged 18 weeks, body weight \( \sim 60 \) kg) were anesthetized and sedated with an intramuscular injection using a combination of Zoletil 100 (tiletamine hydrochloride-zolazepam hydrochloride, 4.4 mg/kg; Virbac Australia Pty Ltd., Milperra, New South Wales, Australia) and Illium Xylazine-100 (xylazine hydrochloride, 2.2 mg/kg; Troy Laboratories Pty Ltd., Glendenning, New South Wales, Australia). They were then intubated, ventilated, and maintained on halothane, oxygen, and nitrous oxide. Pupillary dilation was achieved with tropicamide 1% and phenylephrine hydrochloride 2.5%.

Baseline assessment in all eyes included binocular indirect ophthalmoscopy. After intravenous injection via an ear vein of 10 mg/kg Rose Bengal dye (Sigma Aldrich, North Ryde, New South Wales, Australia), which is a dye with peak absorption of light close to the wavelength of the argon laser and allows an intravascular thrombus to be created with minimal damage to the vessel wall with use of appropriate laser powers. A photothrombotic BRVO was attempted in the vein adjacent to the optic disc in the left eye of each pig using an argon green laser (514 nm wavelength) as described previously.\textsuperscript{32} Immediately after the creation of the BRVO, fluoroconjugated TNK (100 µg/0.1 mL saline) was injected transclerally 2 to 3 mm posterior to the superotemporal limbus via a 30-gauge needle into the midvitreal cavity of both right and left eyes of each pig. The dose of TNK was based on the results from our previous study showing that intravitreal doses below 150 µg produced no evidence of toxicity.\textsuperscript{31} The needle was left in situ for a further 20 seconds after injection before being withdrawn to avoid reflux from the entry site. To ensure the stability of the fluorescent–TNK conjugate, the drug was freshly diluted just prior to injection. TNK (Metylsce; Boehringer, North Ryde, Sydney, New South Wales, Australia) was conjugated with a superior, highly photostable green fluorescent dye, Alexa Fluor 488 (C\(_3\)H\(_7\)CH\(_2\)NH\(_2\)N\(_2\)O\(_2\)S\(_3\)), with excitation/emission maxima at 495/519 nm (custom synthesis of TNK by Molecular Probes, Eugene, OR, for Invitrogen, Mulgrave, Victoria, Australia). The method of conjugation was performed according to that described by Kamei et al.\textsuperscript{27} The Larginine (vehicle) of tPA was replaced with N-acetyl arginine, conjugated with the dye, and thereafter separated with Larginine as the eluting solvent.

Chloramphenical ointment 1% (Sigma Pharmaceuticals Ltd., Victoria, Australia) was applied to the eyes after surgery. After surgery the pigs were allowed to recover before sacrifice 24 hours later. Postoperatively, the animals were kept in a darkened environment to minimize stress and photobleaching. One normal pig eye obtained from the abattoir without injection was used as a control.

The eyes were enucleated and fixed in 4% paraformaldehyde for 24 hours at 4°C, including the control eye. Areas (approximately \( 4 \times 3 \) mm) of vein containing the occlusion from the left eyes, from the same vein of the right eyes with no occlusion, and from the control eye were dissected and embedded in optimal cutting temperature compound (OCT) (Pro Sci Tech Pty Ltd., Thuringowa, Queensland, Australia) for frozen blocks. Enucleated eyes were kept in lightproof jars.

Baseline assessment in all eyes included binocular indirect ophthalmoscopy. After intravenous injection via an ear vein of 10 mg/kg Rose Bengal dye (Sigma Aldrich, North Ryde, New South Wales, Australia), which is a dye with peak absorption of light close to the wavelength of the argon laser and allows an intravascular thrombus to be created with minimal damage to the vessel wall with use of appropriate laser powers. A photothrombotic BRVO was attempted in the vein adjacent to the optic disc in the left eye of each pig using an argon green laser (514 nm wavelength) as described previously.\textsuperscript{32} Immediately after the creation of the BRVO, fluoroconjugated TNK (100 µg/0.1 mL saline) was injected transclerally 2 to 3 mm posterior to the superotemporal limbus via a 30-gauge needle was performed in the left eye. The fellow eye (right) had no injection. Chloramphenical ointment was again applied. The animals were killed 2 weeks later and their eyes enucleated.

Both the TNK-treated and nontreated enucleated eyes had a small slit made just below the limbus and were immediately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer. After 24 hours the eye cup was photographed, and full-thickness sections were cut, stained with toluidine blue for light microscopy (LM), and photographed at oil emersion \( \times 40 \) and \( \times 60 \) magnification. Sections from the area away from the laser
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In group 1 eyes, TEM confirmed the presence of a thrombus composed of engorged stagnant and lysed red blood cells, polymorphonuclearocytes, platelets, fibrin-like material, and debris (Fig. 1). The control abatior eye showed yellow autofluorescence of the pigments in the retinal pigment epithelial layer and faint red fluorescence of the blood cell in the retinal vein with an absence of fluorescence in all other layers of the retina. Green fluorescence of labeled TNK was seen in all the eyes in the lumen of the retinal vein and absent in all other layers. The strongest length of vein occupied by the thrombus was 0.4 mm and the longest 3.98 mm; both of these were seen in the untreated eyes. Histological examination by LM of the thrombus in the untreated eyes revealed stagnant red blood cells, some lysed, fragmented, and/or clumped together and interspersed by polymorphonuclear cells. The vein wall was intact in all eyes, untreated and TNK treated, but was surrounded by macrophages, some of which were laden with pigment granules.

There was no evidence of damage by TNK to the retina (Fig. 4). No difference was discernible under LM between TNK-treated and untreated eyes (Fig. 4). Retinal pigment epithelial cell nuclei were rounded and healthy. Pigment granules appeared typical, dispersed within the cell most toward the apices of the cells. Rod outer segments were mildly disorganized, and some pyknotic cells and swelling of the inner retinal layer and in the ganglion cell layer were also seen in both untreated and TNK-treated eyes. TEM in both eyes showed (Fig. 5) no damaged cells in the retinal pigment epithelial layer. These cells appeared to be active, with apical microvilli engulfing rod outer segments. An occasional pyknotic cell was seen in the outer nuclear layer. A few cells showed vacuolar damage and mild swelling in the inner retina. There was also some swelling in the ganglion cells; however, damaged cells were rare. The cells were endowed with a large number of intracellular organelles including mitochondria, some of which showed mild swelling in both untreated and treated eyes. Inflammatory cells were not seen in any of the eyes examined. TEM confirmed LM findings with no significant difference in necrosis between untreated and TNK-treated eyes (P = 0.357).

RESULTS

Immediately after laser in group 1 and 2 pig eyes, engorgement of the distal vein and scattered intraretinal hemorrhages were seen, indicating that an occlusion had been created. The laser burn was clearly distinguishable on macroscopic examination of the enucleated eye cups in all the eyes in which a vein occlusion was created. In one of the laser-treated eyes in group 1, intraretinal hemorrhage close to the laser burn was clearly distinguishable on macroscopic examination of the distal vein and scattered intraretinal hemorrhages were seen, indicating that an occlusion had been created. The laser burn was clearly distinguishable on macroscopic examination of the enucleated eye cups in all the eyes in which a vein occlusion was created. In one of the laser-treated eyes in group 1, intraretinal hemorrhage close to the laser burn in the eye cup after 24 hours was seen.

In group 1 eyes, TEM confirmed the presence of a thrombus composed of engorged stagnant and lysed red blood cells, polymorphonuclearocytes, platelets, fibrin-like material, and debris (Fig. 1). The control abatior eye showed yellow autofluorescence of the pigments in the retinal pigment epithelial layer and faint red fluorescence of the blood cell in the retinal vein with an absence of fluorescence in all other layers of the retina. Green fluorescence of labeled TNK was seen in all the eyes in the lumen of the retinal vein and appeared to be stronger in the left eyes containing the thrombus as compared to the right eyes without the thrombus. Strong green fluorescence was also seen in the inner limiting membrane (ILM), the vein wall, outer nuclear layer, and rod outer segments in both the left and right eyes with and without the thrombus, respectively (Fig. 2).

In group 2 pig eyes, light microscopic examination of the epoxy resin sections revealed that all six of the untreated eyes had the photocoagulated site of the retinal vein partially occluded with one eye showing extreme narrowing, whereas in the TNK-treated eyes, only one out of the five had a partial occlusion with narrowing of the lumen. The other four treated eyes showed no evidence of an occlusion (Fig. 5) (P = 0.03). ImageJ analysis and computation presented volume of clot of the untreated and treated eyes varying between 0.000426 mm³ and 0.0204 mm³, with volume size significantly greater in the untreated eye (P = 0.028). The percentage blockage of the segment of vein occupied by the thrombus in untreated and TNK-treated eyes ranged between 8.5% and 83.9% (Table). The shortest length of vein occupied by the thrombus was 0.4 mm and the longest 3.98 mm; both of these were seen in the untreated eyes. Histological examination by LM of the thrombus in the untreated eyes revealed stagnant red blood cells, some lysed, fragmented, and/or clumped together and interspersed by polymorphonuclear cells. The vein wall was intact in all eyes, untreated and TNK treated, but was surrounded by macrophages, some of which were laden with pigment granules.

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DISCUSSION

Since the publication of the Branch Vein Occlusion Study (BVO) 25 years ago, grid laser photocoagulation has been the standard of care for BRVO-associated macular edema. In this study, treatment of macular edema following BRVO with grid laser resulted in an average gain of 1 line in vision compared to that in control eyes at the 3-year primary end point. In the last 5 years, several new treatments for macular edema secondary to BRVO have been evaluated in randomized clinical trials. These included intravitreal triamcinolone injections, intravitreal dexamethasone implants, and inhibitor of vascular endothelial growth factor (VEGF) agents. Of these, anti-VEGF agents have been the most promising. In the Ranibizumab for the Treatment of Macular Edema following Branch Retinal Vein Occlusion (BRAVO) study, rapid and sustained visual improvement was seen in patients who received monthly 0.3 mg and 0.5 mg ranibizumab compared with sham injections. At the 6-month primary end point of the study, the mean gain from baseline best-corrected visual acuity (BCVA) letter score was 16.6 and 18.3 Early Treatment Diabetic Retinopathy Study (ETDRS) letters in the 0.3 and 0.5 mg ranibizumab cohorts, respectively, compared with 7.3 letters in the sham group. After 6 months, treatment with ranibizumab on an as-needed basis in all patient groups showed maintenance of the visual gain that was achieved in the ranibizumab groups at the 1-year end point.
**Figure 1.** Photothrombotic branch retinal vein occlusion. (A) Macroscopic view of a normal pig eye indicating the position of the attempted laser photocoagulation (marked). (B) Transmission electron micrograph of an untreated pig eye in group 2, in which a branch retinal vein occlusion was created, showing retinal vein lined by normal-looking endothelial cells (E) with a platelet adjacent to it and lumen occupied by a thrombus containing stagnant red blood cells (RBC), a polymorphonuclear neutrophil (blue arrow) with invaginated nuclei, and phagocytizing cell debris and fibrin-like material (red arrow). Scale bar: 1.4 μm. (C) Transmission electron micrograph of untreated pig eye in group 2, in which a branch retinal vein occlusion was created, showing an area of the thrombus composed of packed stagnant red blood cells (asterisks) and several others that are lysed and fragmented. Scale bar: 1.4 μm. (D) Macroscopic view of the eye cup of an untreated eye showing laser burn site (marked) and attenuation of flow of blood (arrowheads) toward the optic disc (OD). (E) Macroscopic image of the eye cup of a TNK-treated eye showing laser burn site (marked) and flow of blood to the optic disc (OD).

**Figure 2.** Retinal penetration after intravitreal injection of fluorescent-labeled TNK. (A) Epifluorescence micrographs of a frozen section of a normal abattoir pig retina from group 1 eyes taken close to the optic disc in the region of the vein. Faint red autofluorescence of blood cells (arrow) in the retinal vein with all other neural layers devoid of fluorescence is seen. Scale bar: 70 μm. (B) Epifluorescence micrographs of a frozen section of a retina in group 1 pig eye without a created branch vein occlusion and with injected intravitreal fluorescent-labeled TNK, showing staining within the retinal vein (RV) and stronger staining in the vein wall, inner limiting membrane, outer nuclear layer, and rod outer segments. Scale bar: 70 μm. (C) Epifluorescence micrographs of a frozen section of a retina in a group 1 pig eye with a branch vein occlusion created and with injected intravitreal fluorescent-labeled TNK, showing strong staining within the retinal vein (RV), in the vein wall, and inner limiting membrane. Scale bar: 70 μm.
The BVOS group also demonstrated the effectiveness of scatter laser in reducing the development of retinal neovascularization, which is another late sequela of BRVO.\textsuperscript{586} While these results are impressive and represent a major advance in our ability to improve the visual results in this condition, these treatments are all aimed at the sequelae of the condition and make no attempt to directly modify the disease process. BRVOS occur at an arteriovenous crossing, with strong evidence that an intravenous thrombus is the penultimate cause of the occlusion to venous outflow and that there is further active propagation of the thrombus downstream.\textsuperscript{8–10} It is this occlusion that is responsible for the delay in retinal blood flow through the occluded segment and the subsequent VEGF upregulation and elevation in hydrostatic pressure that produce the retinal changes seen clinically.\textsuperscript{3,5} Results from the BRAVO and subsequent extension studies have shown that the VEGF upregulation can be controlled with intraocular injections of ranibizumab with good initial effects on visual acuity; however, injections in many patients need to be continued for years.\textsuperscript{36–39} The other potential component of the macular edema is the elevation of the venous hydrostatic pressure in the occluded segment. This is resolved only once either the clot is recanalized or hemodynamically significant collateral circulation occurs. Collateral vessels have been noted to take between 2 and 18 months to appear after BRVO and may take additional time after this to develop to the stage where they have any significant effect on the elevated venous hydrostatic pressure.\textsuperscript{40} A treatment aimed directly at relieving the obstruction to venous outflow may potentially play a role additional to current conventional anti-VEGF therapies.

Thrombus formation is a dynamic process, and sheer forces, flow turbulence, and platelets in the circulation all can greatly influence the architecture of the thrombus.\textsuperscript{41} In this study there was some variability in the volume size of the thrombus and the distance of vein occupied by it. This variability may be attributed to propagation by further recruitment of clot elements in the larger thrombi or, in the smaller thrombi, the initiation of recanalization and/or clot retraction to renew blood flow.\textsuperscript{42}

The porcine retina is holangiotic and has a vascular supply very similar to that of the human retina. In this study we investigated the ability of a 100 µg intravitreal injection of TNK to penetrate the retina and bind to and lyse an experimentally created intravenous thrombus in a porcine animal model. TNK was shown to penetrate all layers of the retina in the eyes in group 1, with labeled TNK seen in the lumen of the retinal vein in eyes both with and without a BRVO. We have previously also demonstrated that TNK has the ability to penetrate all the layers of the retina 6 and 24 hours after intravitreal injection.\textsuperscript{29} In the eyes with a BRVO, the labeled TNK is seen bound within the clot, showing that this agent has the ability to penetrate the retina and bind to an intravenous thrombus within 24 hours.

In the eyes in group 2, an intravitreal injection of TNK successfully lysed an intravenous thrombus in 80% of the eyes by 2 weeks after the injection compared to the control group ($P = 0.03$); all eyes with a BRVO and no intravitreal TNK showed persistence of the thrombus at the same time point and significantly greater clot volume size ($P = 0.028$).

We have previously investigated the safety of intravitreal TNK to the inner and outer retina, its ability to penetrate the retina, and its ability to effectively lyse subretinal hematomas in animals and humans.\textsuperscript{29–31,43}

Tissue plasminogen activator has been extensively used in humans in an attempt to lyse clots and fibrin collections; however, significant questions remain concerning its effectiveness, potential toxicity, and ability to penetrate the retina. It was originally developed for use in myocardial infarction; however, because of its short half-life, the treatment involved a
bolus injection followed by a two-step 90-minute infusion. The effectiveness of its use intravitreally has also been questioned in the treatment of submacular hemorrhage (SMH). It binds very strongly to the ILM of the retina, which limits its ability to act either within the retina on an intravascular thrombus or underneath on a SMH. In a study on rabbit eyes, labeled tPA was found layered on the retinal surface after an intravitreal injection, with no penetration into the retina. An additional study of tPA retinal penetration by Mahmoud et al. in porcine eyes showed conflicting results. The investigators used an occlusion/reperfusion model of RVO and found some evidence of tPA penetration using an antibody to tPA by immunoperoxidase histochemistry. Owing to variable background staining in their negative controls in the immunoperoxidase staining, they performed indirect immunofluorescence staining of the tPA with conflicting results; no fluorescence staining was seen within the retinal veins either with or without an occlusion. Both methods showed intense staining at the level of the ILM, highlighting the barrier effect that this layer has to retinal penetration by tPA. It remains unanswered if indeed tPA penetrated the lumen of the retinal veins in that study, considering the variability in the background staining in the control samples, the absence of staining in the lumen of the veins by indirect immunofluorescence histochemistry, and no

### Table

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*Figure 4. Effect of intravitreal injection of TNK on retinal histology.* (A) Light micrograph of a group 2 untreated pig retina, section away from the laser burn. The retina looks normal. Retinal pigment epithelium (RPE) appears normal and healthy with rounded nuclei, mildly disorganized rod outer segments (OS), outer nuclear layer (ONL) containing a few pyknotic cells, and mild swelling of cells in the inner nuclear layer (INL) and ganglion cell layer (GCL). Scale bar: 17 μm. (B) Light micrograph of a group 2 pig retina treated with intravitreal injection of TNK, section away from the laser burn showing a normal healthy-looking retina similar to the untreated pig retina, with retinal pigment epithelium (RPE) appearing normal and healthy with rounded nuclei, mildly disorganized rod outer segments (OS), outer nuclear layer (ONL) containing a few pyknotic cells, and mildly swollen cells in the inner nuclear layer (INL) and ganglion cell layer (GCL). Scale bar: 17 μm.
FIGURE 5. Effect of intravitreal injection of TNK on retinal ultrastructure. Transmission electron micrograph from group 2 untreated and treated pig retina, section away from the laser burn showing similar ultrastructure. (A) Untreated, (E) treated: ganglion cell layer including a ganglion cell with nucleus (N) and mitochondria mildly swollen in both eyes. (B) Untreated, (F) treated: inner nuclear layer cells with nuclei (N) showing swelling in some cells in both eyes. (C) Untreated, (G) treated: outer nuclear layer with regularly arranged nuclei (N) in both eyes. (D) Untreated, (H) treated: Retinal pigment epithelium cells appear normal and healthy in both, with pigment granules in the apical region of the cell and microvilli (arrows) engulfing rod outer segments (asterisks) in both eyes. Scale bar: 2 μm.
report of tPA penetration into the other layers of the retina. By comparison the current study, supported by our previous studies, has shown that TNK can penetrate the ILM into all the layers of the retina, evidenced by the strong staining seen in tissue as deep as the rod outer segments.

While tPA and TNK are similar-sized molecules (approximately 70 kDa), TNK in the pig vitreous is neutral or anionic whereas tPA in both rabbit and pig eyes is likely to be cationic. The cationic charge of a molecule can be a limiting factor to diffusion across the retina—most likely due to binding to biomacromolecules, mainly glycosaminoglycans in the retina, especially in the ILM. For anionic or neutrally charged molecules, this is less likely to happen.

Another significant problem with the commercial preparation of tPA is its potential toxicity to the retina, which limits the dose that can be used intraocularly. The toxicity of tPA to the retina has been studied in animal eyes, and toxicity has been found to occur with intravitreal doses equal to or greater than 50 µg. In humans, presumed toxic effects have been reported with doses as low as 50 µg; and as absolutely safe levels have not been established, most studies for the treatment of SMH have used doses between 25 and 50 µg. The retinal toxicity of the commercial preparation of tPA is thought to be primarily caused by the L-arginine within the vehicle. Unfortunately, no commercial preparations of tPA exist without the L-arginine component of the vehicle, and therefore the potential for inducing toxic effects to the retina remains with intraocular use of this agent.

TNK is a third-generation thrombolytic that was developed to address some of the shortcomings of tPA in the treatment of myocardial infarctions. It has a longer plasma half-life than tPA (18 compared to 4 minutes), slower plasma clearance, 14 times the fibrin specificity of tPA, and 80 times more resistance to inactivation via tissue plasminogen activator-1 (PAI-1). TNK, due to its greater fibrin specificity and ability to penetrate the thrombus, requires a significantly shorter clot contact time than tPA to effect thrombolysis. Another significant advantage of TNK is that its vehicle contains less than one-third the L-arginine content than the equivalent dose of tPA.

We have previously investigated the retinal toxicity of TNK to the retina in both rabbit and pigs eyes by clinical follow-up (indirect ophthalmoscope and intraocular pressure measurements), electroretinography, LM, and TEM. In rabbit eyes, intravitreal doses above 100 µg were associated with increased retinal damage. In pig eyes, doses of 50 µg both intravitreally and subretinally showed no evidence of damage to either the inner or outer retina or retinal pigment epithelium. We have also used 50 µg TNK intravitreally to treat SMH in humans and found no evidence of toxicity. In the current study we found no evidence of retinal damage analyzed by LM and TEM in the pig eye, which has a larger vitreous volume than the rabbit eye, following an intravitreal injection of 100 µg TNK. There was no significant difference between untreated and treated eyes (P = 0.357). Inflammatory cells were absent; however, a few damaged and some swollen cells were observed in both treated and untreated eyes, which may be attributed to the effects of transmission of the laser to areas farther away from the burn site and to processing artifact.

Intravitreal TNK may be useful as an acute treatment for RVOs of recent onset. It would have to be considered before any significant thrombus organization and endothelial proliferation occurred. It may potentially improve retinal venous outflow and hasten resolution of retinal edema. While it is unlikely to replace current anti-VEGF therapies, intravitreal TNK may, by improving retinal venous outflow, reduce the current burden of treatment with the multiple injections required over prolonged periods.

A clinical trial to answer these questions, and also address the need for other supplemental medications to prevent reoccurrence of the thrombus in the acute phase, will be required and is currently being planned.

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