Retinal Toxicity of Commercial Intravitreal Tissue Plasminogen Activator Solution in Cat Eyes

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Background: We previously reported retinal toxic reactions in rabbit eyes receiving intravitreal injections of commercial tissue plasminogen activator (tPA) in concentrations greater than or equal to 50 µg/0.1 mL, and recent clinical experience suggests that intravitreal tPA solution may produce toxic effects in human eyes. We therefore investigated the dose-dependent retinal toxicity of intravitreal commercial recombinant tPA solution in cat eyes, which have a vascularized inner retina and vitreous volume similar to that of human eyes.

Methods: Commercial tPA in L-arginine solution was injected into the mid vitreous cavity of normal cat eyes in doses of 25, 50, 75, and 100 µg/0.1 mL and 200 µg/0.2 mL. Control (fellow) eyes received an equal volume of sterile saline solution. After injection, eyes were evaluated by ophthalmoscopy and electroretinography for 14 days and then enucleated for histopathological evaluation.

Results: Fundus pigimentary alterations were observed in eyes receiving doses greater than or equal to 50 µg/0.1 mL. Changes were centered in the area around the injection site, and the area’s size increased in proportion to the dosage. Mean electroretinography B-wave amplitude measured at 14 days was significantly reduced in eyes receiving greater than or equal to 50 µg of tPA in a dose-dependent fashion. Light microscopy of the involved areas showed loss of photoreceptor elements with necrosis and proliferation of the retinal pigment epithelium.

Conclusion: Intravitreal injection of commercial tPA solution results in dose-dependent retinal toxicity in cat eyes. Clinical Relevance: Because cat eyes are similar to human eyes regarding retinal vascularity and vitreous volume, intravitreal injections of commercial tPA (with L-arginine vehicle) in concentrations greater than 25 µg/0.1 mL are potentially unsafe in human eyes.

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MATERIALS AND METHODS

Commercial recombinant human tPA solution (Activase; Genentech Inc., San Francisco, Calif) was reconstituted as directed to a concentration of 1 mg/mL. The vehicle consisted of L-arginine phosphate (34 mg/mL), phosphoric acid (10 mg/mL) and polysorbate 80 (<80 mg/mL). Small aliquots of tPA were maintained at −70°C until immediately prior to use when they were warmed to room temperature and diluted in sterile balanced salt solution (BSS; Alcon Laboratories, Ft Worth, Tex) to concentrations of 25, 50, 75, and 100 µg/0.1 mL.

Institutional guidelines regarding animal experimentation were followed. Domestic short-haired cats weighing 2.5 kg to 4.5 kg were anesthetized with subcutaneous ketamine hydrochloride (4 mg/kg) and xylazine hydrochloride (0.5 mg/kg), and the pupils were dilated with 2.5% phenylephrine hydrochloride and 1% tropicamide. Following baseline indirect ophthalmoscopy and electrotoretinography (ERG), a 30-gauge needle was inserted into the mid vitreous cavity 4 mm posterior to the limbus in the superotemporal quadrant, taking care to avoid contact with the crystalline lens. In each animal, one eye received intravitreal tPA and the fellow eye received BSS as a control. The injection was performed slowly with the needle directed toward the optic nerve and the bevel oriented anteriorly. The needle was held in place for 30 seconds after injection to minimize reflux from the entry site, and indirect ophthalmoscopy was subsequently performed to look for injection complications. Three animals were tested with each dose. Injection volumes were 0.1 mL of tPA or 0.1 mL of BSS, except for the highest dose at which 0.2 mL of tPA or 0.2 mL of BSS was injected. An anterior chamber paracentesis of 0.1 mL was performed in all eyes receiving injected volumes of 0.2 mL. All eyes were confirmed by pneumotonometry to have an intraocular pressure less than 40 mm Hg 5 minutes after injection. Each eye was dressed with dexamethasone-polymyxin-Bacitracin ointment before being returned to the vivarium. All eyes were examined by slitlamp biomicroscopy at 3, 7, and 14 days after injection, and the ERG was recorded on day 14.

Findings from ERG were recorded simultaneously from both eyes of the anesthetized animals using Burian-Allen bipolar corneal electrodes (Hansen Ophthalmic Development Laboratory, Iowa City, Iowa) after corneal anesthesia (0.5% topical proparacaine) and full pupillary dilation (2.5% phenylephrine hydrochloride and 1% tropicamide). The indifferent electrode was a stainless steel needle placed subcutaneously on the back. Signals were amplified at 1000 gain at 0.1 Hz to 1000 Hz (~3 dB points), and a 60-Hz notch filter was used to minimize line noise. The responses were digitized at a 1000-Hz rate, averaged to reduce noise and analyzed off line. A Ganzfield 30-millisecond flash was used, with maximum intensity of 2.3 log candela (cd)/m². Intensity was controlled with neutral density filters covering a 6-log unit range. Animals were dark-adapted for 1 hour prior to beginning scotopic ERG recordings. B-wave amplitudes were measured from the A-wave trough. Statistical comparisons of ERG data were performed using a t test. B- and A-wave amplitudes of treated eyes were expressed as a percentage of the fellow eye to minimize variation between animals. There were no significant differences in mean A- and B-wave amplitude ratios between dosage groups prior to injection.

Euthanasia was performed immediately following the final examination. The eyes were enucleated and refrigerated in phosphate-buffered 2% glutaraldehyde and 4% paraformaldehyde solution after making a slit through the eye wall. Following fixation for 24 to 48 hours, the eyes were hemisected and the temporal hemisphere of each eye was dehydrated, embedded in JB-4 plastic (glycomethacrylate), serial sectioned, and stained with toluidine blue and Lee stain (methylene blue and basic fuchsine) for light microscopy.

With higher doses of tPA, toxic reactions became more apparent. In all of the eyes receiving 75, 100, and 200 µg of tPA, mild to moderate anterior chamber inflammation was present. Inflammatory vitreous debris was visible in 1 eye in the 75-µg group and in 2 eyes in both the 100- and 200-µg groups. One eye injected with 200 µg of tPA developed a more severe vitreitis, presumed (without culture) to represent low-grade endophthalmitis. In all other eyes, the mild inflammatory vitreous debris cleared by 14 days after injection.

Striking fundus pigmentary alterations were noted in 2 eyes in the 75-µg group (Figure 2) and in all 3 eyes in both the 100- and 200-µg groups (Figure 3 and Figure 4). These fundus changes were typically geographic and centered around the injection site. The fundus alterations typically involved a gray motting of the tapetal light reflex by 3 days after injection, with the later development of a non-elevated “cerebriform” pattern of presumed outer retinal folds (best seen in Figure 4), which persisted through the 14-day follow-up period. The involved area was larger with higher tPA doses, and the entire fundus was involved at the 200-µg tPA dose. In eyes receiving the 200-µg tPA dose, widespread vascular attenuation was also apparent by 14 days after injection.

ELECTRORETINOGRAPHY

Mean B-wave amplitudes (stimulus intensity −0.7 log cd/m²) were significantly reduced at doses of 50 µg (P = .04) and above in a dose-dependent fashion (Figure 5). A maximum mean (SD) reduction of 86.8% (9.7%) was found in the 200-µg group (P < .001). Mean B-wave amplitude ratios were not significantly reduced in the 25-µg group.

Mean A-wave amplitudes were also significantly reduced at doses of 75 µg (P < .005) and above in a dose-dependent fashion (Figure 6). A maximum mean (SD) reduction of 69.5% (6.5%) was found in the 200-µg group (P < .001). In the A-wave analysis, this trend was not statistically significant in the 50- or 25-µg groups.

Linear regression across all doses showed a tight correlation between amplitude reduction and dose, with R = 0.988 for the A-wave regression and R = 0.991 for the B-wave regression.

LIGHT MICROSCOPY

All eyes in the 25-µg group were histologically normal and indistinguishable from control eyes by an examiner masked to treatment group (Figure 7). In the 50-µg group, 2 of 3 eyes were histologically normal. In the remaining eye,
outer retinal damage similar to that seen at higher doses was found in a localized area corresponding to the pigmentary alterations noted ophthalmoscopically (Figure 8).

Findings from histopathologic examination revealed retinal cellular damage in all eyes receiving 75, 100, and 200 µg of tPA (Figure 9 and Figure 10). The geographic extent and severity of damage increased with increasing dosage. Photoreceptor outer segments were shortened, and outer retinal folds were seen. Retinal pigment epithelium (RPE) proliferation and necrosis with some pigment clumping was present in affected areas. In correlation with the ophthalmoscopic appearance, the damage was less severe or absent in areas distant from the needle site. There were numerous vitreous polymorphonuclear cells in the eye with presumed low-grade endophthalmitis (200-µg dose), but there was no evidence of retinitis.
This study was engendered by a clinical need to know the retinotoxic threshold for intravitreal commercial tPA solution. Our study demonstrated fundus pigmentary changes in 1 of 3 eyes in the 50-µg group, 2 of 3 eyes in the 75-µg group, and all eyes in both the 100- and 200-µg groups. These changes were most pronounced in the quadrant of injection and involved increasingly larger areas at higher doses. Findings from histopathologic examination showed loss of photoreceptor elements and RPE damage in all eyes with ophthalmoscopic abnormalities, and findings from ERG confirmed functional retinal impairment that roughly corresponded with the results of the histopathologic examination. Significant reductions in mean B-wave amplitudes were first seen with intravitreal injections of 50 µg/0.1 mL. As the injected tPA concentration was increased to 75 µg/0.1 mL and higher, both A- and B-wave amplitudes were significantly and progressively reduced compared with control eyes. Since the dark-adapted ERG A-wave reflects rod photoreceptor activity, these results correlate well with the dose-dependent photoreceptor damage found histopathologically beginning with doses of 50 µg/0.1 mL.

A previous study using rabbit eyes found no evidence of retinal toxicity after intravitreal injections of commercial tPA solution in a dose of 25 µg/0.1 mL. One of 4 eyes injected with 50 µg/0.1 mL showed localized loss of photoreceptor cells, and severe retinal damage was seen in eyes receiving commercial tPA doses of 75 µg/0.1 mL or more. Similar to our findings in the current study, the toxic reaction in rabbit eyes was greatest in the quadrant of injection. The previous study demonstrated that the L-arginine vehicle of the commercially available solution, rather than the tPA protein itself, is the toxic agent. The authors speculated that L-arginine may have toxic effects on the outer retina because of its structural similarity to lysine, an amino acid with known retinotoxic potential. Other investigators have also implicated arginine as a retinotoxic agent. After intravitreal injection in rabbit eyes of commercially available aztreonam, which utilizes an L-arginine vehicle, photoreceptor loss and significant reductions in ERG amplitudes were found. Damage was more severe near the injection site and was nearly as severe after injection of an equivalent amount of the L-arginine vehicle alone. Because these 2 previous reports have implicated L-arginine as a toxic component of commercial pharmacologic vehicles, our current study did not repeat assays com-
paring the retinal toxicity of commercial tPA solution with that of its vehicle alone. Unfortunately, tPA for medical applications is currently unavailable in the United States in preparations that do not contain the L-arginine vehicle.

We were surprised to find that the margin of safety for intravitreal commercial tPA solution in cat eyes was similar to that in rabbit eyes. The vitreous volume of cat eyes is larger than that of rabbit eyes and is estimated to be approximately 70% that of human eyes, based on axial length measurements. However, this larger size conferred little protection from the toxic effects of intravitreal tPA. When coupled with the striking geographic distribution of toxic changes around the injection site, this observation suggests that the tPA solution is not diluted uniformly throughout the vitreous cavity. This may be owing to limited dispersion of tPA solution through the formed vitreous gel of adult cat eyes. It is also possible that tPA solution binds to components of the vitreous and creates a drug depot with prolonged local effect. It has been shown that intravitreal tPA concentrations in nonvitrectomized rabbit eyes 30 minutes after injection are approximately twice as high in the injected quadrant compared with more distant sites. Also, the fact that lensectomy/vitrectomy raises the toxic threshold for tPA in rabbit eyes may be explained in part by greater tPA dilution in liquid vitreous compared with formed vitreous gel. Whatever the mechanism, uneven dilution within the vitreous gel has important implications for human eyes, and suggests that tPA should only be injected in concentrations known to be safe, without the assumption of further dilution.

Limited data are available regarding the toxic effects of tPA in human eyes. Herriot observed no definite signs of retinal toxicity in 20 human eyes injected with intravitreal commercial tPA in doses of 100 µg/0.1 mL to facilitate pneumatic displacement of submacular hemorrhage. Similarly, Hassan et al found no evidence of retinal or other intraocular toxic reactions to tPA solution in 15 patients receiving intravitreal doses of 25 µg/0.1 mL to 100 µg/0.1 mL for the same procedure. Specifically, they observed no retinal pigmentary changes outside areas of previous subretinal hemorrhage, no unexplained visual loss, and no association between tPA dose and visual acuity outcomes. However, Gilbert reported a severe pigmentary retinopathy in a human eye that received an intravitreal tPA injection of 100 µg/0.1 mL. Similarly, Hesse et al recently reported inferior exudative retinal detachment and RPE hyperpigmentation in 4 eyes treated with 100 µg of intravitreal tPA solution, but not in 7 eyes that received 50 µg. Complete preoperative and postoperative ERG recordings in 3 patients revealed a 75% reduction of the scotopic B-wave amplitude postoperatively in a patient treated with 100 µg of tPA and no amplitude reduction in 2 patients treated with 50 µg. Finally, LaPuentet observed mild fundus pigmentary changes after intravitreal tPA injection in concentrations as low as 33 µg/0.1 mL.

Factors affecting the sensitivity of a given human eye to toxic reactions by commercial tPA solution are unknown, but may include vitreous volume, extent of vitreous liquefaction, and position of the needle tip relative to the retina. Although increased fundus pigmentation may partially protect the rabbit retina from the toxic effects of gentamicin, it is not known whether this may also be a factor in human eyes injected with tPA.

In summary, we have demonstrated that intravitreal injection of commercially available tPA solution results in dose-dependent retinal toxicity in cat eyes. This toxic effect preferentially involves the outer retina and RPE. Toxic effects are greatest in the injected quadrant, implying that tPA does not diffuse uniformly throughout the vitreous. Based on these findings, we recommend avoiding intravitreal injections into human eyes of commercially available tPA (with L-arginine vehicle) in concentrations greater than 25 µg/0.1 mL.

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