**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**

**RAD:** 13-159374- -7 **FECHA**: 2014-12-31 09:28:18
**DEP:** 2020 DIRECCION DE NUEVAS CREACIONES **EVE:** 378 FASE NACIONAL INCLUIDO CAPITULO I
**TRA:** 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II **FOLIOS**: 13
**ACT:** 519 PRESENTACION

## DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
GERMAN MARIN RUALES

**Asunto**: Radicación: 13-159374- -7
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 15833
Folios: 13

| | | | | | |
|---|---|---|---|---|---|
| Patente de Invención | **X** | | | Patente de Modelo de Utilidad | |
| Vía Nacional | | | | | |
| Vía PCT (Fase Nacional) | **X** | Capítulo I | **X** | Capítulo II | |
| No. Solicitud Internacional | PCT/IN2011/000863 | | | | |
| Fecha de Presentación Internacional | 16/12/2011 | | | | |

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

### 1. Documentos estudiados

| | | |
|---|---|---|
| Descripción: | Radicado 13-159374-00000-0000 | 05/julio/2013 |
| Reivindicaciones: | Radicado 13-159374-00000-0000 | 05/julio/2013 |
| | Radicado 13-159374-00006-0000 | 03/febrero/2014 |
| Oposiciones | Radicado 13-159374-00003-0000 | 29/octubre/2013 Lafrancol |
| Respuesta del solicitante | Radicado 13-159374-00006-0000 | 03/febrero/2014 |
| Prioridad: | IN 3526/MUM/2010 | 23/diciembre/2010 |

### 2. Análisis de la Prioridad

Se acepta la reivindicación de la prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

### 3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capitulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las

reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso se estudiarán las reivindicaciones 1 a 15 del capítulo reivindicatorio radicado bajo N° 13-159374-00006-0000.

Título de la solicitud: “Composiciones farmacéuticas de tenecteplasa”

El problema planteado en esta solicitud consiste en proporcionar formulaciones de tenecteplasa que tienen una eficacia y seguridad óptimas comparadas con los agentes conocidos de terapias según están identificados en pruebas clínicas de las composiciones.

Para resolver este problema técnico la solicitud en estudio proporciona una composición farmacéutica y kits que comprenden: tenecteplasa, un amortiguador inorgánico y agentes estabilizadores.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 38/16, A61K 9/16 y A61P 9/10.

**4. Excepción a la Patentabilidad** (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 7 – 11 no es patentable de acuerdo con el Art citado. Es oportuno aclarar que aunque las reivindicaciones 7 a 11 estén encabezadas como productos (composiciones y kits) en realidad hace referencia a un método para tratar un accidente cardiovascular con las composiciones reclamadas en las reivindicaciones 1 a 6.

**5. De la solicitud de patente**

**Reivindicaciones** (Art 30 D 486, Art 22 PCT)

Con respecto a las reivindicaciones encabezadas como kit-de-partes este Despacho aclara a la solicitante que un producto tipo kit-de-partes no puede incluir las instrucciones de preparación ni la forma de administración de cada una de las preparaciones. Adicional a ello tampoco puede definirse o caracterizarse en términos exclusivos de la forma de ingesta o administración, el uso clínico, el metabolismo de los fármacos combinados o de los parámetros farmacocinéticos o farmacodinámicos de los compuestos.

Las expresiones utilizadas para definir la composición en la reivindicación 1 “0,10 a 0,24 mg/kg de peso corporal de tenecteplasa” y “para el tratamiento de accidentes cerebrovasculares isquémicos agudos en pacientes humanos” hacen referencia o están asociadas a una dosis y en consecuencia a un método para prevención o tratamiento de una enfermedad, lo que conllevaría a que la materia no sea patentable en Colombia, por lo que requiere a la solicitante para que excluya estás expresiones de la reivindicación 1.

La reivindicación 1 no es clara porque la utilización de dos enlaces gramaticales “que comprende” y “está caracterizada” para una misma reivindicación no permite a la persona del oficio normalmente versada en la materia identificar las características técnicas esenciales de la materia reclamada, es decir aquellas características que definen la invención y la hacen diferente del estado de la técnica y, por lo tanto, constituyen la solución al problema técnico que intenta resolver la invención . Se sugiere a la solicitante presentar las reivindicaciones de acuerdo con la estructura: “Preámbulo - enlace gramatical - parte característica”.

La reivindicación 1 no es clara ni concisa porque la utilización de las expresiones

ellas como “un amortiguador inorgánico aceptable farmacéuticamente” y “un portador aceptable farmacéuticamente” refiriéndose a los elementos constituyentes de una composición no son limitativas y son imprecisas, lo cual no permite distinguir la invención del estado de la técnica. Por lo que se hace indispensable que el solicitante indique la denominación común de los excipientes que cumplen con las condiciones de “un amortiguador inorgánico aceptable farmacéuticamente” y “un portador aceptable farmacéuticamente”

La reivindicación 1 no es concisa porque el término “opcionalmente” empleado en ella es relativo y no permite distinguir la invención del estado de la técnica, se sugiere remplazarlo por un término preciso o un rango.

El alcance de la reivindicación 1 va más allá que el contenido divulgado en la descripción y no permite determinar el efecto técnico, se invita al peticionario modificar la reivindicación 1 para restringir la solicitud a una razonable generalización del tipo de composiciones que ha sido sintetizado ó probado, es decir, que se encuentran debidamente sustentados en la descripción. El siguiente cuadro ejemplifica la afirmación anterior y presenta las composiciones que se encuentran soportados en la descripción.

| **Componentes que se encuentra reclamados en la reivindicación 1 de la solicitud** | **Componentes soportados en la descripción** |
|---|---|
| tenecteplasa | Tenecteplasa<br>1 parte de TNK<br>(0,10; 0,14; 0,18; 0,20 y 0,24 mg/ml)<br>(Pág. 14; Pág. 19, Ejemplo 6) |
| un amortiguador inorgánico aceptable farmacéuticamente | 3,4 partes de ácido fosfórico |
| agentes estabilizadores aceptables farmacéuticamente seleccionados del grupo que consiste de L-arginina y polisorbato; | 11 partes de L-arginina<br>y 0,086 partes de polisorbato 20 |

Las reivindicaciones 1 y 5 no son aceptables porque el uso de las expresiones “en donde dicha composición está caracterizada en que dicha tenecteplasa se prepara por un proceso de fermentación continua basado en la perfusión usando células del ovario del hámster chino” y “en el que los componentes se liofilizan para convertirlos en polvo para su reconstitución con agua para inyección antes de su uso” no corresponde a características técnicas esenciales de una reivindicación de producto tipo composición sino que corresponde a una característica técnica de otra categoría inventiva (procedimiento), lo cual no es admisible puesto que la definición de un producto definido por su proceso de fabricación solo es válida cuando el producto no puede definirse por sus características estructurales y para el caso en estudio las composiciones que se encuentran divulgadas en el capítulo descriptivo puede ser definida por sus fórmulas cuali-cuantitativas, es decir sus características técnicas esenciales, se sugiere a la solicitante definirlo el producto por sus características esenciales, estructurales o funcionales.

La reivindicación 12 no es inadmisible porque la reivindicación hace referencia a la descripción según la expresión “sustancialmente como se describe con referencia a los ejemplos” lo cual es aceptable. Se sugiere eliminar las referencias a la descripción en el capitulo reivindicatorio.

## 6. Análisis de la Oposición

### Argumentaciones de Lafrancol

La opositora manifiesta que las composiciones y formulaciones farmacéuticas que comprenden Tenecteplasa, útiles en el tratamiento del accidente cerebrovascular isquémico agudo son prácticas y eventos totalmente conocidos en la industria farmacéutica, por lo que manifiesta que carecen de los requisitos de novedad y nivel inventivo. Igualmente, indica que la solicitud pretende obtener patente de composiciones farmacéuticas que involucran agentes terapéuticos como Tenecteplasa, señalando que no puede la solicitante pretender beneficios patentarios sobre formulaciones farmacéuticas que comprenden moléculas que se encuentran descubiertas y plenamente identificadas en el sector químico-farmacéutico manifestando que en documentos como “The Index Merck” se evidencia que el principio activo Tenecteplasa se encuentra bajo conocimiento público desde un tiempo considerable y señala como los principios activos Tenecteplasa posee beneficios patentarios bajo el documento WO 9324635.

Finalmente, afirma que la solicitud pretende reivindicar combinaciones, las cuales no se consideran invenciones porque “la mezcla (combinaciones) de productos (moléculas) conocidas y su variación, bien sea en uso, forma, dimensión o materia, NO se consideran invenciones por cuanto no son susceptibles de aplicación industrial, nivel inventivo y novedad, luego NO pueden acceder a beneficios patentarios.

El opositor solicita tener como prueba los siguientes documentos:

| Ítem | Documento | Título | Fecha de publicación* |
|---|---|---|---|
| **D1** | Fifteenth Edición 2006 Página 1694 | Index Merck “An encyclopedia of chemical, drugs and biologicals. | 2006 |

**Respuestas de la Solicitante**

La solicitante manifiesta en cuanto a la observación dirigida a que el principio activo tenecteplasa se encuentra bajo el conocimiento público desde un tiempo considerable, es de señalar que dicho libro The Merck Index proporciona información general sobre Tenecteplasa como una molécula, sin embargo no divulga nada acerca de una composición y/o kit farmacéutico que comprende Tenecteplasa reclamados, por lo concluye que la materia reclamada es nueva frente a las enseñanzas del documento denominado “The Merck Index”.

Por otra parte en cuanto a la alegada falta de novedad de la materia reclamada frente al documento D1

**Respuesta del examinador**

En primer lugar este Despacho aclara que el solicitante presentó un nuevo capítulo reivindicatorio conformado por 12 reivindicaciones, el cual es aceptado puesto que el mismo no implica una ampliación de materia.

Asimismo este despacho informa al opositor sobre la existencia de la modalidad de “invención por combinación” la cual reúne elementos conocidos, pero son en sí una nueva solución técnica a un problema técnico, lo que hace que su afirmación sobre que “la mezcla (combinaciones) de productos (moléculas) conocidas y su variación, bien sea en uso, forma, dimensión o materia, NO se consideran invenciones por cuanto no son susceptibles de aplicación industrial, nivel inventivo y novedad, luego NO pueden acceder a beneficios patentarios” sea imprecisa.

Por otra parte este Despacho aclara que el documento “The Merck Index” no hace

referencia a composiciones o kits que comprendan el activo tenecteplasa, es decir no divulga las características técnicas esenciales de la materia reclamada. Ahora bien con respecto al documento WO 9324635, el mismo no afecta la novedad de la materia reclamada en el capítulo reivindicatorio radicado bajo N° 13-159374-00006-0000 porque aunque el documento revela composiciones que comprenden variantes glicosiladas del activador del plasminógeno tisular T-PA (que incluyen la tenecteplasa), glicerol y un buffer que elaborado con arginina y ácido fosfórico, el mismo no hace referencia a una composición tal y como es ahora reclamada, es decir una composición que comprende tenecteplasa, L-arginina y polisorbato, por lo que este Oficina considera que la materia reclamada corresponde a una selección de dos o más lista de la materia divulgada en el documento citado por la opositora y en consecuencia el documento presentado en el escrito de oposición no puede ser considerado válido para afectar el requisito de novedad.

En conclusión este Despacho informa que los documentos presentados en la oposición no desvirtúan la novedad del objeto de esta solicitud por esta no refiere las composiciones que comprenden tenecteplasa, L-arginina y polisorbato, por lo que considera que la oposición presentada debe ser declarada infundada.

## 7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: diciembre 23 de 2010, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

| Ítem | Documento | Título | Fecha de Publicación* |
|---|---|---|---|
| **D1** | No aplica | "TNKase™ Tenecteplase recombinant" | 11/marzo/2010 |
| **D2** | Br J Ophthalmol 2004;88:573–578. | "Retinal toxicity of intravitreal tenecteplase in the rabbit" | Abril/2004 |

El documento D1[1] divulga un vial que contiene una composición farmacéutica que comprende: 52.5 mg Tenecteplasa, 0.55 g L-Arginina, 0.17 g, ácido fosfórico y 4.3 mg polisorbato 20, que incluye un sobrellenado 5% y en donde cada vial libera 50 mg de Tenecteplasa (Pág. 1, Descripción)

El documento D2[2] divulga una composición farmacéutica que comprende: Tenecteplasa 5mg/ml, L-Arginina (52.2 mg/ml), ácido fosfórico (16mg/ml) y polisorbato 20 (0.4mg/ml) (Pág. 1, Materiales y métodos)

**8. Examen de Patentabilidad** (Art 14 D486)

**Novedad** (Art 16 D486)

La reivindicación 1 consiste en: *"Una composición farmacéutica…"* (Radicado Nº. 13-159374-00006-0000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

1: https://web.archive.org/web/20100311014916/http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/UCM200875.pdf

2: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1772065/

| Características esenciales | D1 | D2 |
|---|---|---|
| Una composición farmacéutica que comprende: | Un vial que contiene una composición farmacéutica que comprende: | Una composición farmacéutica que comprende: |
| tenecteplasa | Tenecteplasa 52.5 mg[3] equivalente a 50 mg | Tenecteplasa 5mg/ml |
| un amortiguador inorgánico aceptable farmacéuticamente | ácido fosfórico 170 mg | ácido fosfórico (16mg/ml) |
| agentes estabilizadores aceptables farmacéuticamente seleccionados del grupo que consiste de L-arginina y polisorbato; | L-Arginina 550 mg y polisorbato 20 4.3 mg | L-Arginina (52.2 mg/ml) y polisorbato 20 (0.4mg/ml) |

Las características esenciales de la composición de la reivindicación 1 habían sido divulgadas previamente en los documento D1 y D2, los cuales revelan composición farmacéutica que comprenden: 52.5 mg Tenecteplasa, 0.55 g L-Arginina, 0.17 g, ácido fosfórico y 4.3 mg polisorbato 20 (D1) y Tenecteplasa 5mg/ml, L-Arginina (52.2 mg/ml), ácido fosfórico (16mg/ml) y polisorbato 20 (0.4mg/ml) (D2)

En consecuencia, la reivindicación 1 no es nueva o carece de novedad, según lo divulgado en los documentos D1 y D2.

Las reivindicaciones dependientes 2, 3, 5 y 6 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas

La reivindicación 4 consiste en: *"Una composición farmacéutica…"* (Radicado Nº. 13-159374-00006-0000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

| Características esenciales | D1 |
|---|---|
| Una composición farmacéutica que comprende: | Un vial que contiene una composición farmacéutica que comprende: |
| tenecteplasa | Tenecteplasa 52.5 mg[4] equivalente a 50 mg |
| un amortiguador inorgánico aceptable farmacéuticamente | ácido fosfórico 170 mg |
| agentes estabilizadores aceptables farmacéuticamente seleccionados del grupo que consiste de L-arginina y polisorbato, en donde el agente estabilizador es una combinación de 11 partes de L-arginina y 0,086 partes de polisorbato. | L-Arginina 550 mg y polisorbato 20 4.3 mg |

Las características esenciales de la composición de la reivindicación 4 habían sido divulgadas previamente en el documento D1, el cual revela una composición farmacéutica que comprenden: 52.5 mg Tenecteplasa, 0.55 g L-Arginina, 0.17 g, ácido fosfórico y 4.3 mg polisorbato 20.

## 9. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

| Ítem | Documento | Reivindicaciones afectadas | Requisito que afecta |
|---|---|---|---|
| **D1** | "TNKase™ Tenecteplase recombinant" | 1-6 | Novedad |
| **D2** | "Retinal toxicity of intravitreal tenecteplase in the rabbit" | 1,2, 3, 5 y 6 | Novedad |

3:cada vial libera 50 mg de Tenecteplasa

4:cada vial libera 50 mg de Tenecteplasa

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

**JOSÉ LUIS SALAZAR LÓPEZ**
**Director de Nuevas Creaciones (c)**

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-01-06

Elaboró: Lenin Roberto Guerrero
Revisó: Consuelo Leguizamon Leguizamon

EXTENDED REPORT

# Retinal toxicity of intravitreal tenecteplase in the rabbit

**S A Rowley, S Vijayasekaran, P K Yu, I L McAllister, D-Y Yu**

*Br J Ophthalmol* 2004;**88**:573–578. doi: 10.1136/bjo.2003.027466

See end of article for authors' affiliations

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Accepted for publication 1 September 2003

**Aim:** To investigate the retinal toxicity of intravitreal injection of a novel fibrinolytic tenecteplase in rabbit eyes.

**Methods:** Tenecteplase (25–350 μg in 0.1 ml BSS) was injected into the vitreous cavity of normal rabbit eyes. Control (fellow) eyes received 0.1 ml of BSS. One day, 1 week, and 2 months post-injection, the eyes were examined by slit lamp biomicroscopy, indirect ophthalmoscopy, and electroretinography, and then harvested for histopathological examination.

**Results:** No evidence of retinal toxicity was seen with tenecteplase doses up to and including 50 μg. At a dose of 150 μg ophthalmoscopy was normal, but histology showed mild retinal damage in the inner nuclear layer and electroretinography showed a temporary reduction in B-wave amplitude. At doses of 200 μg and above, there was evidence of retinal toxicity on electroretinography, ophthalmoscopy, and histology. Ophthalmoscopic findings included vitreal fibrosis, retinal necrosis and tractional retinal detachment and light microscopy revealed necrosis of retinal pigment epithelium and other retinal layers. Damage was centred around the injection site but was more widespread with the higher doses.

**Conclusion:** A dose of 50 μg tenecteplase appears safe for intravitreal injection in the rabbit. Tenecteplase could have potential applications in the treatment of submacular haemorrhage and retinal vein occlusion.

Tissue plasminogen activator (tPA) is a recombinant plasminogen activator which binds to the fibrin component of thrombus and converts thrombus bound plasminogen to plasmin which initiates local fibrinolysis. Although primarily used in the management of acute myocardial infarction, it has also been used for various indications in the field of ophthalmology. These include dissolution of fibrin from the surface of intraocular lenses,[1] accelerating the resolution of post-vitrectomy fibrin,[2] treatment of central retinal vein occlusion with local[3] and systemic treatment,[4] and facilitating clearance of submacular haemorrhage.[5]

Shortcomings of tPA in the management of acute myocardial infarction include short half life necessitating infusion rather than a bolus injection[6] and this has led to the development of modified tPA compounds such as tenecteplase. Compared to tPA, tenecteplase has a longer half life and greater fibrin specificity, and so has potentially greater efficacy in thrombus dissolution.[7] This could provide significant advantages in ophthalmic applications.

The results in animal studies of intravitreal injection of tPA in concentrations greater than 25 μg/0.1 ml have implied that use of this dose of tPA in humans would be unsafe.[8 9] As tenecteplase may have a better outcome in ophthalmic use, we therefore investigated the retinal toxicity of intravitreal injections of various doses of tenecteplase using slit lamp examination, indirect ophthalmoscopy, electroretinography (ERG), and light microscopy.

## MATERIALS AND METHODS

The tenecteplase used in this study was the commercially available preparation and was donated by the manufacturer (Metalyse; Boehringer Ingelheim, NSW, Australia). The undiluted drug after reconstitution had a concentration of 5 mg/ml. To establish the toxicity profile of the vehicle, a stock solution was prepared consisting of L-arginine (52.2 mg/ml), polysorbate 20 (0.4 mg/ml), and phosphoric acid 85% (16 mg/ml) (product information, Boehringer Ingelheim). As the stability of cryopreserved solutions of tenecteplase has not been established, the drug was freshly reconstituted and diluted to the appropriate concentration each time surgery was performed.

### Surgery

The rabbits were anaesthetised using an intramuscular combination of ketamine hydrochloride (35–50 mg/kg) and xylazine hydrochloride (3–5 mg/kg). The pupils were dilated with 2.5% phenylephrine hydrochloride and 1% atropine. Following baseline ERG, the retina was examined before injection using a planoconcave contact lens and operating microscope. A 30 gauge needle was introduced into the vitreous cavity 2 mm posterior to the nasal limbus and under direct vision with the contact lens, the needle tip (bevel anterior) was positioned just above the retinal surface 1.5 disc diameters below the inferior edge of the optic disc. A volume of 0.1 ml of the test solution was then administered. The needle was held in place for 10–15 seconds after injection before withdrawing to prevent reflux from the entry site. The central retinal artery was observed to be patent after each injection. The fellow eye of each animal was used as a control and injected with 0.1 ml of BSS. Chloramphenicol ointment 1% (Chirosig, Sigma Pharmaceuticals Ltd, Victoria, Australia) was applied to the eyes immediately after surgery.

A total of 35 New Zealand White rabbits weighing 2–4 kg, were used in this study. To establish the dose levels to be tested for the main study a pilot study using 13 of the rabbits was conducted and the tenecteplase was diluted in sterile balanced salt solution (BSS; Cytosol Ophthalmics Inc, Lenoir, USA) to give doses of 25, 50, 100, 200, 250, and 350 μg. As part of this pilot study, vehicle equivalents of all these tenecteplase doses were also prepared as previous work has established that the toxicity of tPA is caused by the vehicle.[8]

After the dose levels had been established, the main study was performed using tenecteplase doses of 50, 150, 200, and 250 μg. In all, 22 rabbits were used for this study, six being used for 50 μg and 250 μg and five being used for 150 μg and 200 μg.

### Clinical follow up

All the eyes were examined by slit lamp biomicroscopy, and indirect ophthalmoscopy 1 day, 1 week, and 2 months post-injection. ERG and intraocular pressure measurement (Tonopen, Mentor Ophthalmics, MA, USA) were also recorded at each examination.

### Electroretinography

Photopic ERG recording was carried out on anaesthetised rabbits using a Nicolet Compact Four electroretinography suite and Ganzfeld stimulation (Nicolet Biomedical Instruments, WI, USA) with background light set to level 2. The reference electrodes were made of silver wires electroplated with chloride. The electrodes were threaded into the scalp and lateral canthus of the rabbit head.

The corneal electrode was a contact lens electrode (ERG-jet, Universo sa, Switzerland). Amplifier bandpass was 1–1000 Hz, and the stimulus interval was 0.9 seconds. Each reported ERG is the average of 32 consecutive flashes. Using four increasing stimulus intensities of 0.5, 0.75, 1.0, and 1.50 log units, four averaged ERGs were recorded for each eye at every time point. Data were recorded at four time points; immediately before the injection of tenecteplase, then 1 day, 1 week, and 2 months after the injection. B-wave amplitude was measured from the A-wave trough for each recording (fig 1).

### Histology

After final examination by ophthalmoscopy and electroretinography, the animals were killed with lethal injection of pentobarbitone and the eyes enucleated. Each eye had a small slit made in the region of the pars plana and was immediately placed in 2.5% glutaraldehyde in 0.1 M phosphate buffer. After 30 minutes the anterior segment was removed. Three days later, the eye cup was vertically bisected. One half was dehydrated in graded series of ethanol and processed in paraffin (Paramat) (BDH, England). Several pieces of tissue from the other half, approximately 2×3 mm were excised from the optic disc, medullary ray, inferior and superior retina, and from the cornea and processed for epoxy resin (Durcapan ACM, Fluka AG, Switzerland). The pieces were first post-fixed in osmium tetroxide, then dehydrated in graded series of ethanol, infiltrated with and embedded in epoxy resin. Sections of 4 μm paraffin and 1 μm epoxy were cut and stained. Paraffin sections were stained with haematoxylin and eosin, and epoxy sections with toluidine blue for light microscopic examination.

### Statistics

Statistical analysis of intraocular pressure and ERG data was performed using paired *t* test. B-wave amplitudes of different time points after tenecteplase administration were expressed as a percentage of that before tenecteplase administration to minimise the effect of inter-animal variation. Statistical significance was determined as p value equal to or less than 0.05.

All experimental procedures were carried out in accordance with the animal experimentation ethics committee of the University of Western Australia.

## RESULTS

### Clinical follow up

#### Tenecteplase and control eyes injections

None of the 35 control eyes injected with 0.1 ml BSS developed posterior segment inflammation or damage. Two of the control eyes had mild anterior chamber activity on day 1, which settled after 1 week.

The eye injected with 25 μg tenecteplase showed no sign of anterior or posterior chamber inflammation. Of the eight eyes injected with 50 μg, one eye was excluded because of retinal touch during injection. One of the remaining eyes had a hypopyon and mild vitritis on day 1 which resolved after a week, and was thought to be the result of iatrogenic lens touch. Another eye had a mild vitritis on day 1 which again had resolved by 1 week. The remaining five eyes injected with 50 μg tenecteplase showed no evidence of anterior or posterior chamber inflammation. The eye injected with 100 μg tenecteplase showed no signs of anterior or posterior chamber inflammation.

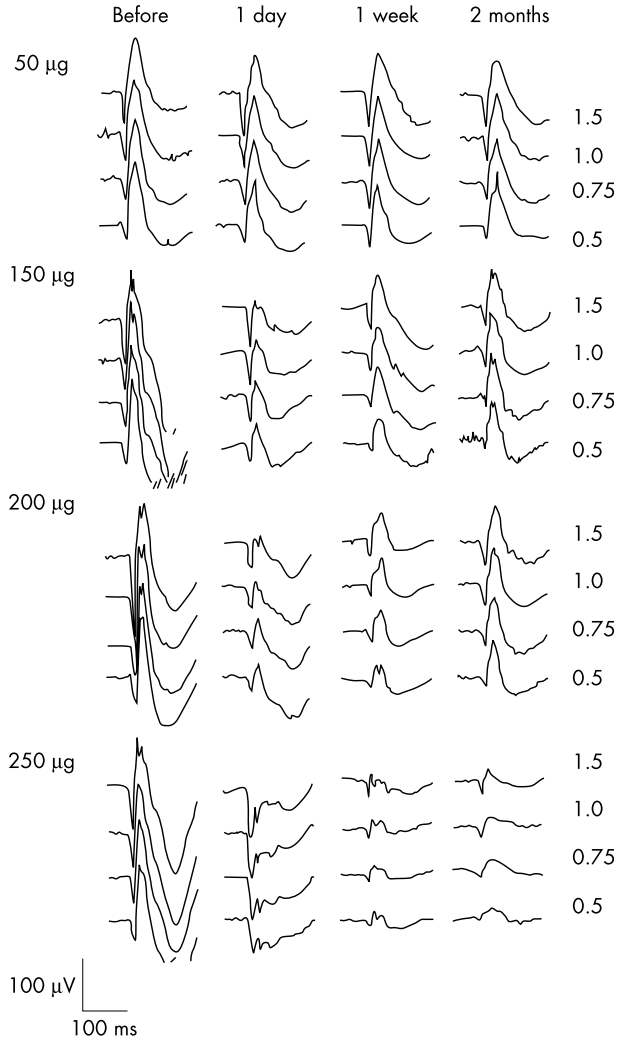


**Figure 1** Typical ERG recordings of rabbit eyes before, 1 day, 1 week, and 2 months after exposure to 50, 150, 200, and 250 μg tenecteplase. Results from all four strobe intensities (0.5, 0.75, 1.0, and 1.5) are presented for each time point. Scale bar shows 100 μV in the y-axis and 100 ms in the x-axis.

None of the five eyes injected with 150 μg tenecteplase showed any sign of anterior or posterior chamber inflammation. One of the eyes had a preretinal haemorrhage inferior to the disc at day 1 but this was resolved at 1 week.

Four of six eyes injected with 200 μg showed moderate vitritis at day 1. Of these four eyes, two eyes progressed to tractional retinal detachment and in one eye areas of retinal atrophy developed inferior and superior to the optic disc. The third eye with initial vitritis had normal fundal examination at 2 months. One eye was ophthalmoscopically normal at all examinations and one rabbit died before the 2 month follow up. There was no anterior chamber inflammation visible in any of the eyes.

The most severe toxicity was seen ophthalmoscopically in the eyes injected with 250 and 350 μg tenecteplase. All of the eyes developed vitritis at day 1. Four of the eyes also had fibrous vitreal condensations and two eyes had areas of retinal oedema. At 1 week, four eyes progressed to traction retinal detachments and three had areas of retinal necrosis. The remainder had fibrous vitreal bands. At 2 months the areas of tractional retinal detachment had increased, one eye had also developed an area of retinal atrophy, two eyes had areas of retinal necrosis, and four had fibrous traction within the vitreous.

In the eyes with retinal pathology, the area affected to the greatest extent was around the site of injection—that is, inferior to the optic disc.

### Vehicle injections

The eyes injected with the vehicle equivalent of the doses above showed no anterior or posterior chamber reaction at doses up to 100 μg equivalent. The 200 μg dose initially showed no sign of inflammation but at the 2 month follow up, vitreal fibrosis and an area of retinal atrophy were apparent inferior to the optic disc. With the 250 μg and 350 μg doses, vitritis was present from day 1 and both eyes developed traction retinal detachments at 2 months.

### Intraocular pressure

There was no statistical difference in intraocular pressures between control eyes and eyes receiving injections of tenecteplase at any of the doses tested (table 1).

## Electroretinography

Before injection the average B-wave amplitude was 158 (8.1) μV at 0.5 log stimulus intensity; 167 (8.6) μV at 0.75 log; 177 (9.1) μV at 1.0 log; and 190 (9.5) μV at 1.5 log stimulus intensities. There was no significant reduction in B-wave amplitude with 50 μg tenecteplase ($p>0.2$) (table 2). At 150 μg tenecteplase, however, there was a drop in B-wave amplitude to 75.6% of original value by day 1 ($p<0.01$). The drop was even more severe at higher doses of 200 and 250 μg where only 51% and 39.6% of the original B-wave amplitude was seen 1 day after injection ($p<0.01$) (fig 2). Although this drop recovered by 1 week in the 150 μg group, only partial recovery was seen in the 200 μg group to 68% ($p<0.01$) by 2 months, and no recovery was seen in the 250 μg group (40.1% of original B-wave amplitude at 2 months, $p<0.01$). Regarding the doses of tenecteplase with limited data points and the eyes tested with vehicle equivalent doses, B-wave amplitude showed no reduction at 25 μg but at doses of 100 μg and above, there was a dose dependent reduction in amplitude.

## Histology

Light microscopic examination correlated to ophthalmoscopic and electroretinographic findings and revealed the presence of visible damage to the retina with higher doses (200 and 250 μg) of tenecteplase. In most of these eyes, damage was predominantly in the inferior retina, medullary ray, and optic disc. The eyes that received 25, 50 (fig 3A), 100 μg tenecteplase and 25, 50, and 100 μg vehicle were indistinguishable from their respective control eyes, which appeared normal. The retinae of all these eyes had healthy looking cells in well organised layers.

However, in the eyes that received 150 μg tenecteplase, minor changes in the inner nuclear layer were observed. Slight swelling of some cells close to the outer plexiform layer was seen (fig 3B). Other layers of the retina appeared normal.

In the eyes that received 200 μg tenecteplase, significant retinal damage was seen in most regions of the inferior retina in all the animals. In the affected areas the retina had lost most of its layered structure and was reduced in thickness. The inner limiting membrane was intact. However, the inner nuclear layer had undergone post-necrotic changes resulting in atrophy of its cells. Vacuoles were also present. Photoreceptor cells had also atrophied and outer and inner segments were absent. Pigment epithelial cells appeared lean and scanty with loss or reduction of its lipid globules (fig 3C).

All the eyes that received 250 μg tenecteplase (fig 3D) showed more severe damage than those eyes that received 200 μg tenecteplase. Post-necrotic atrophy of retinal layers causing extreme reduction in thickness with infiltration of chronic inflammatory cells were noted. The changes were seen in most areas of the inferior retina. In these areas, although the inner limiting membrane was intact, large number of red blood cells and chronic inflammatory cells were present in the vitreous. The cells in the different layers of the retina were greatly reduced with a loss of inner and

**Table 1** Effect of dose and duration of intravitreal tenecteplase on intraocular pressure

| | Mean IOP (SE) (mm Hg), post injection | | |
|---|---|---|---|
| Tenecteplase dose (μg) | 1 Day | 1 Week | 2 Months |
| 0 | 15.03 (0.75) | 15.23 (0.63) | 17.14 (0.76) |
| 50 | 14.57 (1.29) | 14.85 (2.78) | 14.43 (2.12) |
| 150 | 11.00 (2.81) | 14.80 (1.64) | 16.40 (2.08) |
| 200 | 10.50 (1.35) | 13.83 (0.44) | 15.40 (1.15) |
| 250 | 14.00 (2.93) | 12.29 (1.47) | 13.86 (1.91) |

**Table 2** Ophthalmoscopic, electroretinographic, and histological findings with increasing doses of intravitreal tenecteplase

| | Tenecteplase dose (μg) | | | |
|---|---|---|---|---|
| | 50 (n=7) | 150 (n=5) | 200 (n=5) | 250 (n=7) |
| Ophthalmoscopy | Normal | Normal | TRD(2)<br>RA(1)<br>Normal(2) | TRD(3)<br>RN(2)<br>VF(2) |
| ERG B-wave amplitude | No reduction | Transitory reduction | Significant reduction | Significant reduction |
| Retinal histology | Normal | Mild damage | Significant damage | Significant damage |

TRD = tractional retinal detachment; RA = retinal atrophy; RN = retinal necrosis; VF = vitreal fibrosis.

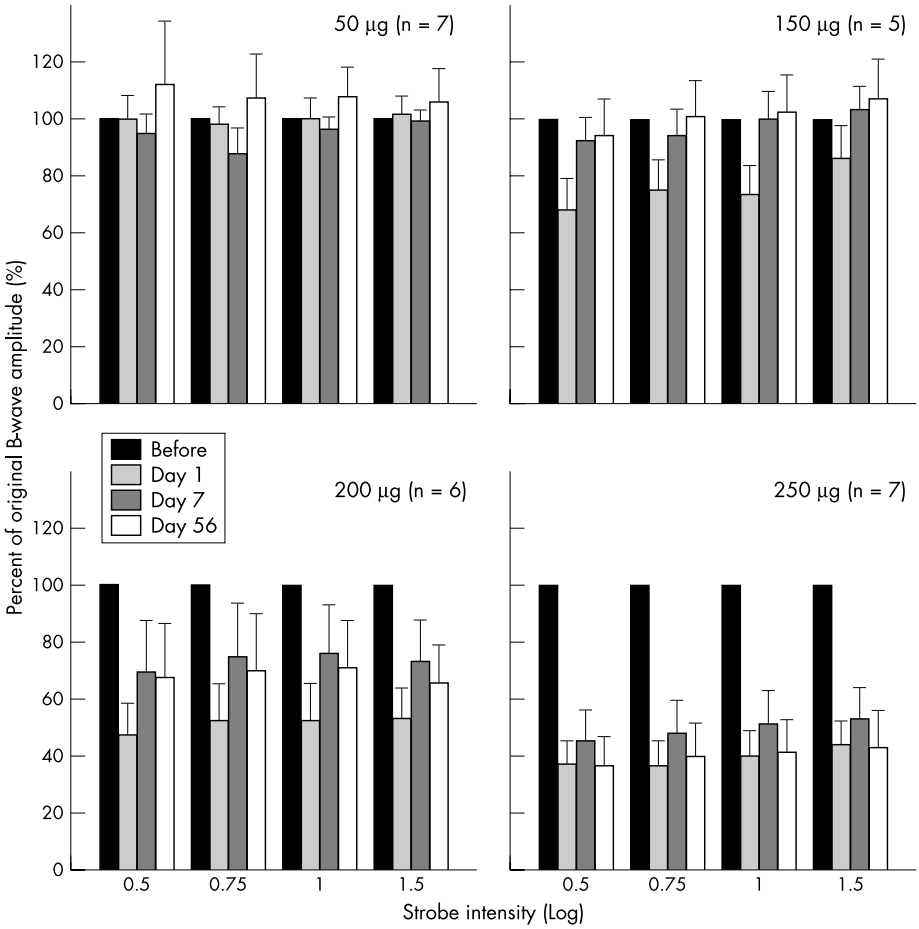


**Figure 2** Graph showing the effect of 50, 150, 200, and 250 µg tenecteplase on B-wave amplitude measured before, 1 day, 1 week, and 2 months after the injection. B-wave amplitude at all stimulus intensities (0.5, 0.75, 1.0, and 1.5) are presented as a percentage of original B-wave amplitude.

outer retina including rod inner and outer segments and vacuoles were present. The lamellar architecture of the retina was completely destroyed and replaced by glial cell proliferation. Chronic inflammatory cells in the retina were also seen. Pigment epithelial cells had shrunk or extruded lipid globules vacuolation and appeared to be undergoing necrosis.

The most severe cases were in the eyes that received the 250 µg and 350 µg vehicle, in which there was full thickness necrosis with the loss of most of the retina except for a remnant thin strand of cells.

## DISCUSSION

Tissue plasminogen activator (tPA) has been widely used in the treatment of acute myocardial infarction but because of the short half life of tPA its administration requires a bolus injection followed by a two step 90 minute infusion.[7] Newer thrombolytic agents such as tenecteplase have been developed to improve on the characteristics of tPA by substituting three single amino acids at the T, N, and K domains.[10] This has resulted in a compound with a longer half life in the systemic circulation (18 minutes compared to 4 minutes) enabling bolus administration, potentially improving the lytic rate by exposing the clot to a rapid high concentration of enzyme.[7 10 11] Tenecteplase also has 14 times the fibrin specificity of tPA, which theoretically improves potency and allows enzymatic activity to occur preferentially at the clot rather than in the peripheral circulation reducing the risk of systemic fibrinolysis.[7 10 11] Tenecteplase is also 80 times more resistant to inactivation by plasminogen activator inhibitor-1 (PAI-1), an enzyme secreted by platelets that inhibits thrombolytics.[7 10 11]

In the field of ophthalmology, tPA has various indications for use, the most common being displacement of submacular haemorrhage. Given the potential advantages of tenecteplase over tPA, tenecteplase may have greater efficacy in the management of this condition and also in the management of central retinal vein occlusion. However, the retinal toxicity profile of tenecteplase needs to be established. The toxicity of tPA has previously been studied in the rabbit and cat[8 9] and established that evidence of retinal toxicity occurred with intravitreal doses greater than or equal to 50 µg. Thus, our data suggest that tenecteplase is less toxic to the rabbit eye than tPA. In human eyes, a dose of 25–50 µg has been commonly used clinically[5 12–14] but exudative retinal detachment has occurred with a dose of 100 µg[13] and fundus pigmentary change with a dose of 33 µg.[15] The toxicity of tPA is thought to be due to the L-arginine component of the vehicle as vehicle equivalents of the tPA doses used in the above work caused identical retinal toxicity.[8] Further evidence comes from a study of intravitreal injections of aztreonam (also containing L-arginine in the vehicle), which showed almost equivalent retinal damage when a vehicle equivalent to the aztreonam dose was injected.[16]

Our study has demonstrated no signs of tenecteplase induced retinal toxicity at intravitreal doses up to and including 50 µg.

There was no sign of tenecteplase induced toxicity in fundus appearance at tenecteplase doses up to 150 µg. However, the ERG showed transient suppression at this dose at day 1 post-treatment. Histopathological examination revealed minor changes in the inner nuclear layer mainly in the cells close to the outer plexiform layer.

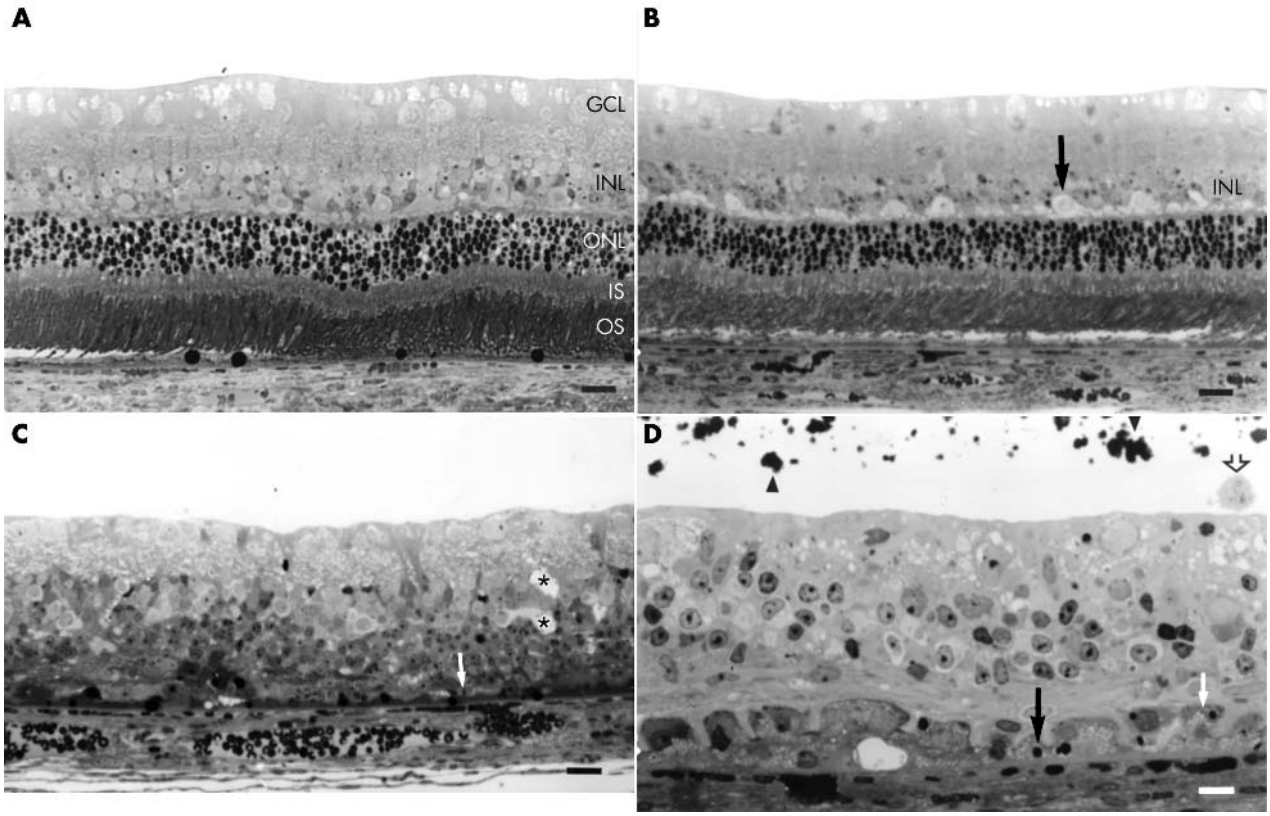


**Figure 3** (A) Light micrograph of a retina, 2 months after intravitreal injection of 50 μg tenecteplase in 0.1 ml balanced salt solution. Retinal architecture of all layers of the retina including rod outer (OS) and inner segments (IS), outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL) appear to be normal (stained with toluidine blue, magnification, scale bar = 20 μm). (B) Light micrograph of a retina, 2 months after intravitreal injection of 150 μg tenecteplase in 0.1 ml balanced salt solution. Mild swelling of cells in the inner nuclear layer (INL) can be seen (arrow). Other layers of the retina appear to be normal (stained with toluidine blue, magnification, scale bar = 20 μm). (C) Light micrograph of a retina, 2 months after intravitreal injection of 200 μg tenecteplase in 0.1 ml balanced salt solution. Significant damage consisting of pigment epithelial cell necrosis (white arrow), post-necrotic atrophy of all layers of the neural retina including rod outer and inner segments, outer nuclear layer, inner nuclear layer and ganglion cell layer causing reduction in thickness. Vacuoles are also seen (*) (stained with toluidine blue, magnification, scale bar = 20 μm). (D) Light micrograph of a retina, 2 months after intravitreal injection of 250 μg tenecteplase in 0.1 ml balanced salt solution. Severe damage of the retina with pigment epithelial cell necrosis and localised proliferation (white arrow) with shrunken lipid globules (arrow) is seen. Post-necrotic atrophy of all layers of the neural retina including rod outer and inner segments outer nuclear layer, inner nuclear layer, and ganglion cell layer causing thinning and gliosis is also seen. Degenerated red blood cells (arrowhead) and a macrophage (open arrow) in the vitreous are present (stained with toluidine blue, magnification, scale bar = 10 μm).

At a dose of 200 μg tenecteplase, significant reductions of B-wave amplitude were noted and histopathological examination revealed significant retinal damage in most regions of the inferior retina in all eyes. There was necrosis of pigment epithelial and retinal cells, post-necrotic atrophy of cells causing thinning of the retina, and gliosis. Despite these ERG and histopathological findings, some eyes displayed no ophthalmoscopic evidence of retinal toxicity.

With tenecteplase doses of 250 and 350 μg the ophthalmoscopic evidence of retinal damage was more severe with the majority of the inferior retina being affected. This was confirmed histopathologically, with damage occurring distant to the site of injection and also on ERG with the greatest reductions in B-wave amplitude being in this group.

Although the vehicle components for commercially available tPA are similar to that of tenecteplase, the concentration is significantly different. A vial containing 10 mg tPA contains 348 mg of L-arginine—that is, 34.8 mg L-arginine/1.0 mg tPA, whereas a vial containing 40 mg of tenecteplase has 417.6 mg of L-arginine—that is, 10.44 mg L-arginine/1.0 mg tenecteplase (product information, Boehringer Ingelheim). Thus for equivalent doses, tenecteplase has less than one third of the L-arginine content than with tPA. We found that the toxicity profile of the vehicle alone was identical to the equivalent tenecteplase dose. This gives further evidence that the retinal toxicity of these compounds is primarily caused by the vehicle.

Although we have found that 50 μg tenecteplase injected intravitreally causes no evidence of retinal toxicity, it should be noted that our study was performed on phakic rabbits with formed vitreous. The rabbit retina is predominantly avascular and the vitreous volume is smaller than that of humans, so it is difficult to extrapolate these results for tenecteplase use in humans. The structurally similar tPA however has shown the same toxicity profile in cats and rabbits, despite the cat having a larger vitreous volume and a vascularised retina[8 9] and the use of intravitreal and subretinal tPA at 50 μg/0.1 ml has shown no signs of toxicity in humans.[12–14] Given that tenecteplase contains less L-arginine dose for dose compared with tPA and has the same molecular weight, one would expect that 50 μg tenecteplase would not cause retinal toxicity in humans.

Other unanswered questions at present are whether the theoretical advantages of tenecteplase over tPA translate into greater clinical efficacy in the resolution of submacular haemorrhage and whether its increased potency will cause problems such as increased rates of re-bleeding. In vitro studies showed a 7.5-fold increase in potency of tenecteplase

compared with that of tPA with respect to lysis of whole blood clots in rabbit arterial venous shunts.[10] Clinical studies showed no significant difference in coronary artery reperfusion rates of tenecteplase and tPA[17] and a large multicentre trial (ASSENT-2) showed equivalence of tenecteplase and tPA in 30 day mortality rates in the treatment of acute myocardial infarction.[18] Regarding the potential risk of rebleeding, the ASSENT-2 trial found similar rates of intracranial haemorrhage and significantly less non-cerebral bleeding events in the tenecteplase group compared to tPA.[18]

In summary, we have demonstrated that intravitreal injection of a novel fibrinolytic, tenecteplase, results in dose dependent retinal toxicity in phakic, non-vitrectomised rabbit eyes. There were no signs of retinal toxicity at doses up to and including 50 μg tenecteplase. Further work is under way to determine if tenecteplase has the potential for greater efficacy in the treatment of submacular haemorrhage and central retinal vein occlusion.

## ACKNOWLEDGEMENTS

The authors would like to thank Judy Granger for laboratory assistance. Grant support was provided by the National Health and Medical Research Council of Australia, grant number 211901.

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