• Basic Research •

Effect of etanercept on post-traumatic proliferative vitreoretinopathy

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Abstract

• AIM: To evaluate the safety and efficacy of intravitreal etanercept in the inhibiting of proliferative vitreoretinopathy (PVR) in a model of penetrating ocular injury.

• METHODS: Penetrating ocular injury on the retina of rabbit was induced, which was subsequently treated using 0.1 mL of sterile water or 0.1 mL of 12.5 mg/mL etanercept. The development of PVR was evaluated by fundus images, the B-scan, and the histopathology. The mRNA and protein expressions of tumor necrosis factor-α (TNF-α), transforming growth factor β (TGF-β) as well as connective tissue growth factor (CTGF) were examined at various time points after the etanercept injection with the reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting, respectively. The safety of etanercept was evaluated by injection of 12.5 mg/mL etanercept into a normal rabbit eye without penetrating trauma.

• RESULTS: Clinical assessment and grading clearly demonstrated that the PVR formation was prevented in etanercept-treated animals, which was confirmed *via* fundus images, B-scan and histopathology. The RT-PCR and Western blotting showed increased mRNA and protein expression of TNF-α, TGF-β as well as CTGF in the retina of rabbits following penetrating ocular injury, and these factors were dramatically mitigated by ocular etanercept treatment. In addition, there was no adverse effect of etanercept intravitreal injection in normal eyes without penetrating trauma, it showed normal structure and histology.

• CONCLUSION: The etanercept is a potential therapy for inhibiting PVR development. To assess the clinic application of the etanercept in preventing PVR, further clinical studies are required.

• KEYWORDS: etanercept/rhTNFR-Ig; traumatic proliferative vitreoretinopathy; TNF-α inhibitor/anti TNF-α; rabbit

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INTRODUCTION

Proliferative vitreoretinopathy (PVR), which occurs in 40%-60% of patients with open globe injuries, is the primary factor in causing recurrent retinal detachment following retinal reattachment surgery and tractional retinal detachment after ocular trauma[1]. PVR is a severe, vision-threatening disorder. PVR will induce worse vision in eyes and is wherefore considered as the primary cause of vision loss[2]. Despite advances in therapeutic approaches and vitreoretinal surgery for PVR management, PVR remains an intractable clinical problem[3]. The exact mechanisms responsible for PVR remain unclear, but it is widely accepted that in its pathogenesis, inflammation is significant[4]. Ocular trauma causes increased inflammation and cytokine production, which increases the occurrence of PVR[5]. Fundamental research on the pathogenesis of PVR on cytokines and growth factors provide types of therapeutic target for blocking the cellular events that contribute to this disease.

Tumor necrosis factor-α (TNF-α), which is a very prominent inflammatory cytokine, is secreted in response to trauma, infection, and inflammation[6]. It is a key mediator of ocular inflammation and its interactions with the retinal pigment epithelium (RPE) cell that initiates PVR[7]. TNF-α was found to have a causative role in PVR as it can act on the RPE and wherefore consequently induces changes in cellular morphologies into fibroblastic cells. Additionally, if TNF-α is combined with other growth factors, a strong synergistic effect can be induced to form epithelial-mesenchymal transition (EMT)-associated fibrotic focus. Etanercept (rhTNFR-Ig, Enbrel®), a TNF-α inhibitor, can bind to TNF-α and therefore functions as a decoy receptor. It was the first biological disease-modifying antirheumatic drug (bDMARD) and the first fusional monoclonal antibody against TNF-α in immune systematic disease, such as juvenile idiopathic

arthritis, psoriatic arthritis or plaque psoriasis, ankylosing spondylitis, and rheumatoid arthritis[8-9]. For patients with noninfective autoimmune uveitis, etanercept is a key cost-effective treatment option[10]. Etanercept could play a potential role preventing inflammation changes involved in the PVR, which remains unknown. This study utilized an animal model to assess the effect of etanercept in the treatment of trauma-induced PVR.

MATERIALS AND METHODS

Ethical Approval The rabbits, kept in individual houses under standard conditions, were handled as per the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. The experiments were specifically approved and overseen by the Care and Use of Laboratory Animal Committee of Tianjin Medical University and Chengdu University of Traditional Chinese Medicine. Routine clinical evaluation and ophthalmic examination were carried out prior to the experiment.

Animals The experiments were performed on 56 male chinchilla rabbits, weighed 2.0-3.5 kg and 2-3 months old. Dashuo Laboratories Ltd. (Chendu, China) provided us with the animals, which were acclimatized for 1wk prior to the experiments.

Groups and Traumatic Proliferative Vitreoretinopathy Model The rabbits were equally and randomly divided into normal group (n=8), PVR model group with sterile water-treated and etanercept-treated group (n=24). Levofloxacin eye drops (0.5%, Santen, Japan) were used three days before and after the intravitreal injection and surgical procedure. The left eyes of normal group rabbits were intravitreal injected with 0.1 mL of sterile water. To assess the security of intravitreal injection of etanercept, the right eyes of normal group rabbits were injected intravitreally with 0.1 mL (12.5 mg/mL, diluted with sterile water for injection) etanercept under general and topical anaesthesia. Traumatic PVR was induced in rabbit of PVR model group and etanercept-treated group as described follows. PVR model group received an additional intravitreal injection of 0.1 mL sterile water 72h after the sclerotomies were sutured. The left eye of etanercept-treated group rabbits were received an additional intravitreal injection of 0.1 mL etanercept (12.5 mg/mL, diluted with sterile water for injection) 72h after the sclerotomies were sutured. The concentrations were chosen based on the preliminary experiment. EU Directive 2010/63/EU and NIH publication No.85-23, edition 1985, were followed in the animal experiments.

Surgical Procedure of Modeling Traumatic Proliferative Vitreoretinopathy To induce traumatic PVR, a series of surgical procedures were performed on the rabbits' eyes under general and local anesthesia. General anesthesia was performed *via* intravenous injection of the 2 mL/kg body-weight pentobarbital sodium (3%), under local anesthesia with oxybuprocaine hydrochloride drops (Benoxil, 4 g/L, Santen, Japan). Tropicamide and phenylephrine (Mydrin) were used to maximally dilate the pupils. To exclude pre-existing fundus abnormality, preoperative fundus examinations were performed. Only the left eye was used in PVR model group and etanercept-treated group, as the experiment procedure might impact the vision of the animals. No trauma was induced on the right eye. The aseptic techniques principles and preoperative care were applied. In this study, surgery was performed *via* an operating microscope by the same surgeon. An oblique scleral incision of 6-8-mm full-thickness was made, at a fixed distance of 3-5 mm, at the upper lateral quadrant of the eye behind the limbus. The vitreous spontaneously prolapsed *via* the wound and wherefore ruptured the anterior vitreous face. After a time of 4h, the prolapsed vitreous was excised. The wounds were subsequently sewed by two or three interrupted 8-0 vicryl sutures. Totally 0.4 mL of autologous blood was injected into the mid-vitreous *via* a 30-gauge needle inserted through the wound under ophthalmoscopic control. The blood was drawn *via* the ear vein immediately before the surgery. The conjunctiva was closed subsequently by means of two 8-0 vicryl sutures. TobraDex ointment (Alcon®) was applied for one week on a daily basis. During the first week postoperatively, a clinical examination was performed on a daily basis. After the first week, clinical examinations were performed weekly. The eyes were enucleated on 10, 20, and 30d after surgery under both general and local anesthesia followed by euthanasia (overdose with pentobarbital sodium).

Ophthalmic Examination Regular ophthalmic examinations were performed 1, 3, 7, 10, 20, and 30d after intravitreal injection. An indirect ophthalmoscopy was used to observe signs of intraocular inflammation, vitreous hemorrhage, haze, or retinal detachment. A slit lamp biomicroscope was used to observe the anterior segment, as well as any signs of inflammatory responses or uveitis. Corneal or lens opacity was examined. Intraocular pressure (IOP) and pupillary light reflex were checked.

Fundus Photography and B-scan The fundus was imaged using the digital fundus camera (Nidek, AFC-330, Japan) 10, 20, and 30d after intravitreal injection. Rats cataract or endophthalmitis were excluded from the protocol. The B-scan ultrasonography (ACCUTOME, USA) was performed simultaneously. Proliferative responses were assessed in accordance with these scales: 0, no proliferative response; 1, vitreous haze, vitreous strands; 2, epiretinal membrane formation with retinal folds; and 3, white dense membrane covering the retina with localized retinal detachments and retinal folds[11].

Histopathological Examination Two rabbits in PVR model group, etanercept-treated group (10, 20, and 30d after trauma respectively) and two rabbits normal group (15d after intravitreal injection) were randomly selected and euthanized. The left eyes of PVR model group and etanercept-treated group, both eyes of normal group were enucleated, fixed in 10% formalin, all of which were then embedded in paraffin at room temperature. The paraffin-embedded eyes were cut into sections of 4-μm horizontally-thickness *via* the eye optic disk, which were subsequently fixed on slides. Hematoxylin-eosin (HE) was utilized to stain the sections to observe the abnormal blood vessel growth, the presence of retinal fold, the epiretinal membrane formation, as well as the disruption in the inner retina (from the inner plexiform layer to the inner limiting membrane). A biological microscope (MoticBA400Digital, Fujian, China) was used to perform microscopic analysis at 40× magnification.

Reverse Transcriptase-Polymerase Chain Reaction Analysis Six rabbits in PVR model group, etanercept-treated group (10, 20 and 30d after intravitreal injection respectively) and six rabbits in normal group (15d after intravitreal injection) were randomly selected and euthanized. The left eyes were enucleated. Vitreous and retinal were dissected and flash frozen in liquid nitrogen, which was then stored in -80℃ refrigerators for further testing. Vitreous and retinal homogenates were prepared. The total RNA was extracted with a Reagent of TRIZOL (16596-026, Invitrogen Life Technologies, ThermoFisher, China) following standard protocol. Amplification of the cDNA was conducted by 35 polymerase chain reaction (PCR) cycles (melt at 95℃ for 30s, anneal at 55℃ for 30s, extend at 72℃ for 1min), with Platinum TaqDNA polymerase (Invitrogen). Trials were carried out to determine proper cycles, so that the amplification did not plateau, but was in the exponential range. The following specific primers were used: TNF-α (F: 5′-CCAGGTTCTCTT CAGCGGTCAAGGC-3′, R: 5′-ACTCGGCAAGGTCCAG GTACTCAGG-3′), connective tissue growth factor (CTGF) (F: 5′-ATGCTGCGAGGAGTGGGTGTGGA-3′, R: 5′-GCCG ACAGAAGGCGTTGTCATTGGT-3′), transforming growth factor-β (TGF-β) (F: 5′-ATAGTCTTCCTACCGGTCCT-3′, R: 5′-TGGGGAGCGTTATGTGCCAG-3′) in SYBR Premix Ex Taq II Kit (RR820A, Takara, Japan) in a real-time PCR cycler (PIKORed 96, ThemoFisher, MA, USA). Relative mRNA level of every sample was determined following the step in which DD CT method was used to normalize the GAPDH mRNA expression.

Western Blotting Tissue was lysed with RIPA lysis buffer (1:10) and lysate was incubated on ice for 15min, then was subject to centrifugal clarification at 14 000×g, 4℃, for 10min (TGL-16G-A, Anke, Shanghai, China). BCA reagents (Thermo Scientific) were used to determine protein concentration. The supernatant was run on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, which was then transferred to a nitrocellulose membrane. Blocking solution [10 g skim milk in 20 mL 1 mol/L Tris-buffered saline and 150 mL 0.1% Tween 20 (TBST)] was used to incubate the membranes for 1h at room temperature, which were subsequently incubated with primary antibody to TNF-α, TGF-β, CTGF, followed by anti-IgG horseradish peroxidase-conjugated secondary antibody. Enhanced chemiluminescence (ECL, BL520A, Thermo, USA) was used to detect the blots. Quantity One software (Bio-Rad, Richmond, CA, US) was used to quantify each band density, which was then normalized to β-actin. All immunoblot analyses were repeated in triplicate, to obtain similar results.

Statistical Evaluation The quantitative data were expressed as the means±standard deviation (SD). Enumeration data were calculated by incidence rate. ANOVA test was used for intragroup statistical comparisons. And the analysis unpaired *t* test was used to determine the significance of the difference with comparisons. SPSS software (version 17.0, Chicago, USA) was used. Significant difference was defined as less than 0.05.

RESULTS

The Safety of Etanercept in the Vitreous No adverse effect was observed in the right rabbits' eyes of normal group following intravitreal injection of etanercept. At 3, 7, or 15d following injection, no sign of retinal detachment, haze, or vitreous hemorrhage was found. Clear transparent anterior segment was demonstrated by a slit lamp biomicroscope in the etanercept-treated eyes, showing no signs of evidence of uveitis or inflammatory response. There was no corneal or lens opacity. The direct and indirect pupillary light reflex was normal. Significant difference was not found in the IOP (mean±SD) after the intravitreal injection of 0.1 mL of 12.5 mg/mL etanercept on the 3rd (8.03±0.54 mm Hg), 7th (8.11±0.76 mm Hg), and 14th (7.98±0.85 mm Hg) day between the left (normal eyes with sterile water-treated) and right eyes (normal eyes with etanercept-treated) in normal group and the pretreatment baseline values (8.23±0.66). And the images of HE staining showed normal histologic retinal tissue, there was no difference between the both eyes of normal group.

Fundus Images and B-scan Showed Etanercept Prevented Traumatic Proliferative Vitreoretinopathy in the Animal Model Fundus examination was normal in the left eyes (Figure 1A) and right eyes (Figure 1E) of normal group. Different grades of PVR were observed in PVR model group. Fundus photographs of the injured eye demonstrated injuries in the retina, which caused vitreous haze, vitreous strands, and epiretinal hemorrhage 10d after the injury (Figure 1B).

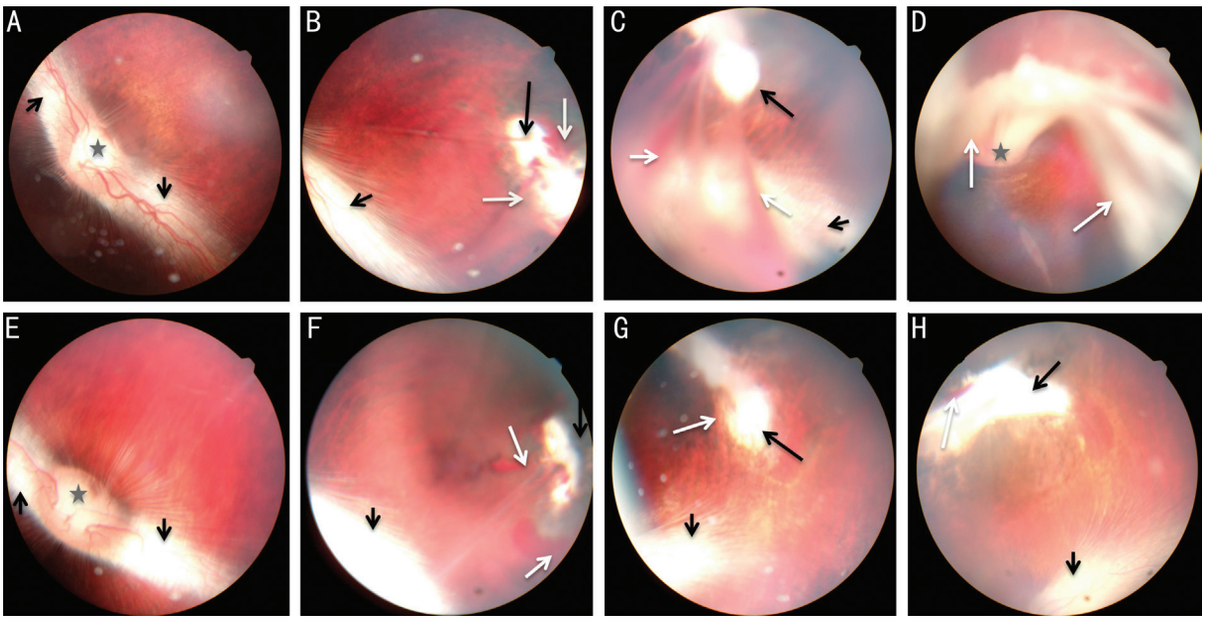

Figure 1 Ocular fundus photographs of rabbit eyes with penetrating ocular injury and etanercept treatment A: Normal rabbit eye shows normal merangiotic vasculature; B: Rabbit eyes after 10d of injection shows epiretinal haemorrhage (white arrows: around the wound); C: After 20d of injection, rabbit eye shows vitreous haze, vitreous strands, and epiretinal membrane being mingled with haemorrhage (white arrows); D: After 30d of injection, rabbit eye shows magnificent retinal detachment and folding (white arrows); E: Intravitreal injection of etanercept into normal eyes shows no abnormalities; F-H: Etanercept treatment shows reduced retinal hemorrhage (white arrows), without formation of thick epiretinal membrane, and retinal detachment. Asterisk denotes optic nerve head, black short arrow denotes the myelinated fibers, and black long arrow denotes the wound of injury.

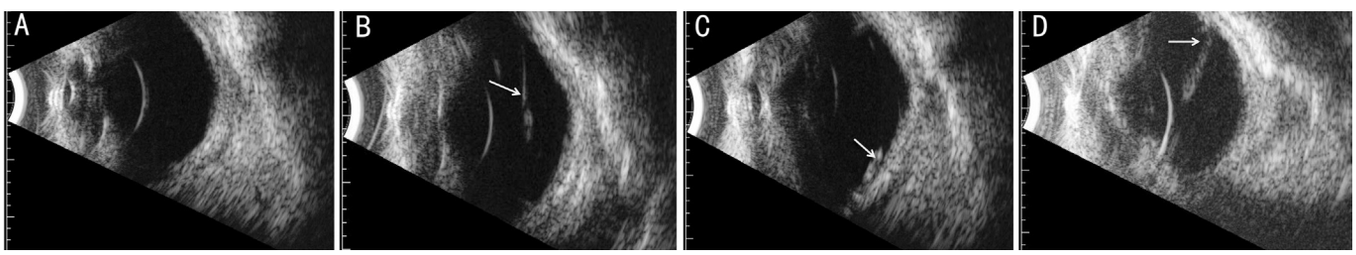

Figure 2 Grading of PVR development by B-ultrasonic measurement in rabbit eyes with penetrating injury A: There is no vitreous haze or abnormal echo in normal rabbits eyes; B: Vitreous haze and strands (arrow) was obvious in rabbit eyes developed stage 1 PVR, but no retinal detachment was seen; C: Vitreous haze, epiretinal membrane formation (arrow) was detected in rabbit eyes developed stage 2 PVR; D: Obvious retinal detachment was observed in rabbit eyes developed stage 3 PVR.

Epiretinal membrane which covered the retinas by localized retinal detachments and retinal folds were observed 20d after the injury (Figure 1C). Epiretinal membrane formation, wrinkling of the retina, and extensive retinal detachment were observed 30d after injury (Figure 1D). The fundus photography of the rabbits in etanercept-treated group did not experience these severe changes. Epiretinal hemorrhages and mild vitreous haze were observed 10d after injury (Figure 1F). Slight focal contraction and moderate vessel tortuosity, with no apparent retinal detachment or severe retinal folds, were observed in the eyes (Figure 1G and 1H). Examinations of PVR model group and etanercept-treated group demonstrated a varied degree of PVR, with cyclitic membrane formation induced by retinal traction up into the wound.

We performed B-scans in all rabbits and noted changes in vitreous and folding of the detached retina. In stage 0, images of B-scan were normal (Figure 2A). The vitreous haze was obvious in stage 1, and funicular echo was not observed (Figure 2B). The funicular echo connected to the retina was detected in stage 2 and local detachment was not seen (Figure 2C). The surface of the retinal was not smooth in stage 3, with significant vitreous traction and retinal detachment (Figure 2D).

Proliferative responses were evaluated according to fundus images and B-scan. An eye which presented with stage 2 or greater was considered retinal detachment, whose incidences were 0 in normal group, 2 (25%) on 10d after intravitreal injection in PVR model group (P-10d), 6 (75%) on 20d (P-20d), 7 (87.5%) on 30d (P-30d); 0 on 10d after intravitreal injection in etanercept-treated group (T-10d), 2 (25%) on

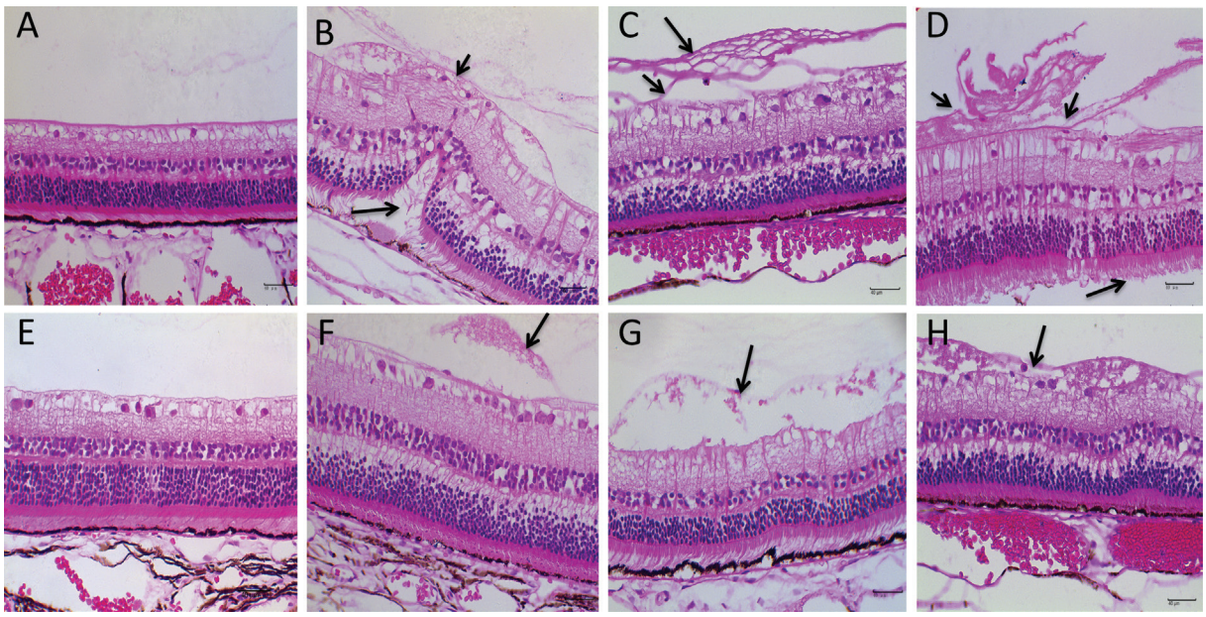

Figure 3 Histopathological evaluation of experimental PVR in rabbit eye (40×) A: Eyes in normal group showed normal HE staining; B: PVR model group on 10d after injection. Vitreous haze, malalignment of internal limiting membrane (short arrow) and local retinal folds (long arrow) are observed; C: PVR model eyes on 20d after injection show epiretinal membrane thicken (long arrow), traction of internal limiting membrane (short arrow); D: PVR model group on 30d after injection, epiretinal fibrous is obviously thickening, retinal traction (short arrow) and separation with loss of the retinal pigment epithelial-photoreceptors interface (long arrow) was observed; E: Normal eyes after etanercept intravitreal injection, the retinal histology is normal; F-H: The signs of fibrotic changes were absence in etanercept-treated group. Vitreous strands and traction (arrow) on the retina is slight, the smooth surface of retina was absent.

Table 1 Frequencies of PVR development in normal group, PVR model group (P), and etanercept-treated group (T) at different time points *n* (%)

PVR stage	Normal	P-10d	P-20d	P-30d	T-10d	T-20d	T-30d
Stage 0	8 (100)	0	0	0	0	0	0
Stage 1	0	6 (75)	2 (25)	1 (12.5)	8 (100)	6 (75)	5 (62.5)
Stage 2	0	2 (25)	4 (50)	1 (12.5)	0	1 (12.5)	2 (25)
Stage 3	0	0	2 (25)	6 (75)	0	1 (12.5)	1 (12.5)

20d (T-20d), and 3 (37.5%) on 30d (T-30d) (Table 1). The difference among the three groups at different time points was statistical significance (P=0.014). Significant differences were found in the retinal detachment rate between PVR model group and etanercept-treated group at different time points (P=0.027).

Histopathology Demonstrated PVR Prevention in Etanercept-treated Eyes The retinas were normal and fully attached in the left (Figure 3A) and right eyes (Figure 3E) of normal group, where the photoreceptor layer and the RPE were intact. PVR model group had HE-stained frozen sections with clearly marked proliferative membranes as well as the retinal detachment between the sensory retinas and RPE, along with destructed retinas. The PVR process was observed in PVR model group at different time points after injury. And HE staining of retinal tissue section showed different pathological changes according to the grades. In stage 1, attached retina, but epiretinal membrane was began to form and local retinal fold was observed (Figure 3B). In stage 2, epiretinal membrane, retinal detachment, the damaged photoreceptor layer and the RPE, and the normal arrangement of the inner nuclear layer is lost (Figure 3C). In stage 3, epiretinal membrane was thickening and obvious traction, retinal detachment was significant (Figure 3D). The retinal folds, detachment, or epiretinal membrane formation was absent in etanercept-treated group, but the smooth surface of retina was absent (Figure 3F) and there was posterior vitreous detachment. The localized retinal separation mimicked pathological retinal detachment, however there was no obvious pathological cause (like fibrosis) (Figure 3G and 3H). The histological eyes sections further supported the clinical observations.

High Expression of TNF-α in Traumatic PVR and Etanercept Downregulates the Expression of CTGF and TGF-β Reverse transcriptase-PCR (RT-PCR) was conducted in this research to assess the expression of the TNF-α and the CTGF. Quality RNA was obtained from six samples in

each group. The retinal and vitreous samples were positive for TNF-α, CTGF and TGF-β mRNA expression *via* RT-PCR testing. Significant increases in the expressions of TNF-α (Figure 4A), CTGF (Figure 4B) and TGF-β (Figure 4C) were observed at various time points in the vitreoretinal tissue from the injured rabbit eyes. Significant (P=0.000) decreases in the mRNA expressions of CTGF (Figure 4B) and (Figure 4C) were observed in etanercept-treated eyes. But there was no difference (P>0.05) in the mRNA expression of TNF-α (Figure 4A) of etanercept-treated group compared with PVR model group.

Etanercept Decrease the Protein Expression of TNF-α, CTGF and TGF-β TNF-α, CTGF and TGF-β in retina and vitreous were checked with Western blotting (Figure 5A). Protein of all these factors had strong relative expression on PVR model group and etanercept-treated group compared with normal group (P<0.05) at all time points after trauma. And treatment of etanercept suppressed the expression of TNF-α (Figure 5B), CTGF (Figure 5C) and TGF-β (Figure 5D) compared with PVR model group rabbits at all time points (P<0.05). Collectively, these results demonstrate that etanercept can directly blind TNF-α protein and inhibit the further biological effect of it. This preventation is associated with the etanercept therapeutic effect on PVR.

DISCUSSION

PVR is an inflammatory fibrotic disease characterized by proliferation and migration of ectopic cells in the periretinal and/or vitreous area when the trauma or retina is broken. Formation of the membranes traction in periretinal areas causes rhegmatogenous retinal detachments[12-13]. The dynamic wound-healing process following tissue injury, including modulation of scar, proliferation, and inflammation, either takes an abnormal path with protracted wound healing, or goes through normal ocular wound healing with tissue remodeling and repair[14]. TNF-α, a cytokine which can promote inflammation, was studied as a promoter of PVR[15]. TNF-α was found in epiretinal membranes of patients and the research animals with PVR, with positive TNF-α staining both intracellularly and in the extracellular matrix[16-18]. TNF-α plays a causative role within PVR, as it can act on RPE and wherefore induces variations among the cellular morphology into fibroblastic cells. It seems that an interplay exists between matrix proteins, growth factors, and various cytokines[19]. The combination of TNF-α and TGF-β1, fibroblast growth factor (FGF), and CTGF has strong synergistic effects that induces EMT associated fibrotic focus formation[20-21]. This fundamental understanding aids to identify various adjunct agents blocking the cell events which are intrinsic to the PVR[14]. Subcutaneous etanercept was the first bDMARD, which is now commercially available as the first TNF inhibitor approved to cure rheumatic diseases[9].

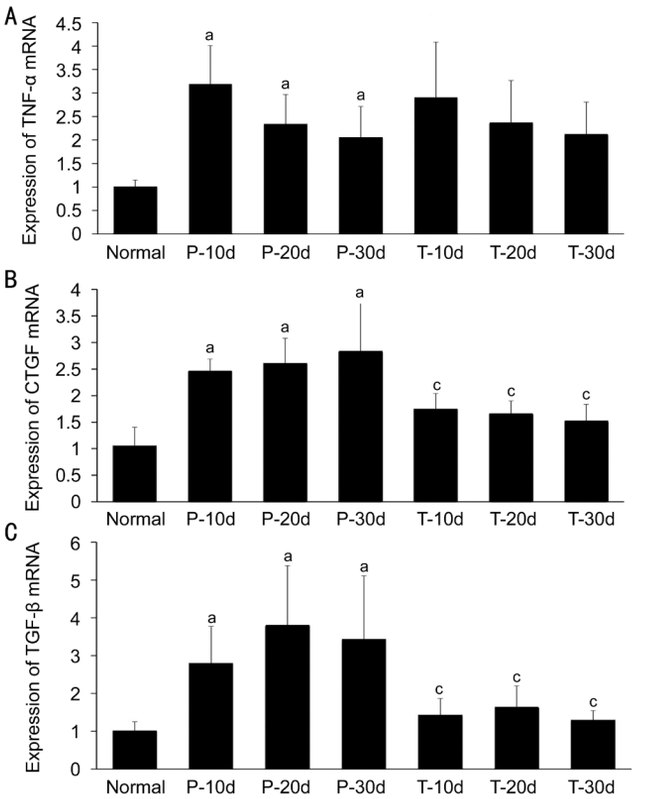

Figure 4 Intravitreal injection of etanercept inhibits TNF-α, CTGF and TGF-β mRNA expression (RT-PCR) A: Bar diagram shows significantly increased TNF-α mRNA expression in PVR eyes at different time points compared with normal eyes. Difference expression of TNF-α mRNA was not found between PVR groups; B: Bar diagram shows significant increased CTGF mRNA expression in PVR eyes at different time points as well as the prevention in etanercept-treated eyes; C: Bar diagram demonstrates significantly increased TGF-β mRNA expression in PVR eyes at different time points as well as the prevention in etanercept-treated eyes. PVR model group (PVR, sterile water-treated) (P) and etanercept-treated group (PVR, etanercept-treated) (T) eyes at different time points (10d, 20d, 30d after injection). Data expressed as mean±SD, n=6. ^{a}P<0.05 *vs* Normal, ^{c}P<0.05 *vs* PVR model group.

We established a PVR model in rabbits, in which intravitreal injures were induced. To clinically mimic the PVR etiopathogenesis, the PVR model was developed by inciting retinal injury on the upper lateral quadrant of the eye behind the limbus at a fixed distance (3-5 mm), which was commonly met in clinical settings. In most cases, traumatic patients cannot receive therapy or operations immediately, where operation is typically performed a few hours later. The suture was performed at 4h after trauma.

Etanercept reduces the inflammatory effect of TNF-α and is used clinically to treat several autoimmune diseases. The TNF-α is shown to take a key role in development of many ocular diseases, *e.g.* diabetic retina ischemia[22], retinopathy[23], glaucoma[24], and retina degenerative diseases[25], where anti TNF drug is a potential therapy for diseases. The ocular

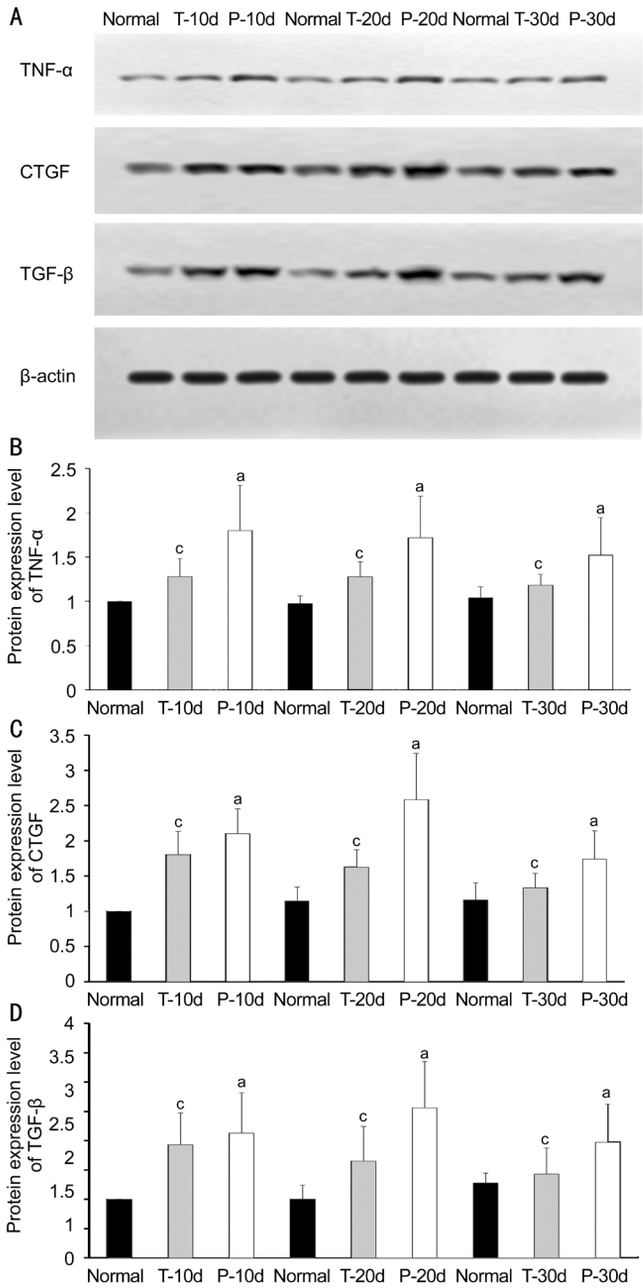

Figure 5 Etanercept inhibits TNF-α and decrease the protein expression of CTGF and TGF-β (Western blotting) A: Protein bands of TNF-α, CTGF and TGF-β. There is significant increased protein expression of TNF-α (B), CTGF (C) and TGF-β (D) in PVR eyes at different time points as well as the decrease in etanercept-treated eyes. All experiments were performed in triplicate. Data expressed as mean±SD. ^{a}P<0.05 *vs* Normal, ^{c}P<0.05 *vs* PVR model group.

topical use of a TNF-α inhibitor was thought to circumvent these adverse systemic side effects. Adalimumab injecting (a TNF-α inhibitors, at a dose up to 2.5 mg) into vitreous bodies of healthy rabbits, is not toxic to the rabbit retina[26]. There were no side effects observed of etanercept (12.5 mg/mL) in the intravitreal injection. Systemic or topical infection was an absolute contraindication for the TNF-α inhibitors, so we performed the vitreous injection three days after trauma to exclude endophthalmitis. Early treatment of etanercept blocked retinal damages induced by trauma and wherefore maintained a more intact retinal structure than the saline-treated rabbits. The retina structure seemed to be stable 10d after the trauma, but inflammation was initiated, which was demonstrated in the retinal tissue in the strong expression of TNF-α. This inflammatory environment caused apoptosis as well as appearances of fibroblastic cells, which could consequently result in severe retina damage over time. The TNF-α provoked morphological variations in the RPE, which demonstrated a gain of spindle cell morphology and a lack of intercellular junctions. The early blockage of the local inflammatory signs *via* etanercept could preserve cellular viability against apoptosis and therefore decreased the expression of TNF-α and CTGF, thus rendering the retinal structure more intact. The TNF-α and CTGF were the predominant cytokines as well as a growth factor in the PVR pathogenesis. The early modulation of the inflammation *via* etanercept likely reduced the potential for PVR development in animals. Recent research states that CTGF is expressed locally in PVR eyes[27]. In this study, expression of CTGF mRNA was evaluated, which demonstrated that, compared to normal rabbit retina, CTGF mRNA expression was increased in the PVR retina.

The results in this work demonstrate that etanercept can be potentially utilized in intravitreal therapy as a secure method to prevent PVR induced by ocular injuries. To assess the clinical application of etanercept, further clinical study is required.

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