

Pathological Changes in Rabbit Retina and Its Relationship with Glutamic Acid after Injuries from High-Speed Bullets

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Abstract

Purpose: To observe the pathological changes in rabbit retinas and the measure of glutamic acid levels in the vitreous body after suffering from high-speed bullet injuries.

Methods: Rabbits eyeball contusion models were established with high-speed bullets, i.e., the rabbits eyes were shot with a fixed air rifle at a speed of 90 m/s (using plastic bullets, weighing 0.201 g, on average). Retinal tissues treated with HE staining and were prepared for light microscopy examination and glutamate levels were tested at different time points after the injury.

Results: Edema, exudation, hemorrhage, and rupture were evident in rabbit retinas following bullet injuries. Meanwhile, glutamate levels gradually increased as time proceeded.

Conclusion: Visual impairment is related with retinal damages after high-speed bullet injuries. Increased glutamate concentration serves as a potential factor for aggravating retinal injuries. (*Eye Science* 2011; 26:239–243)

Keywords: high speed; gunshot injury; pathological change; glutamic acid

Ocular contusions caused by high-speed objects have been regarded as one of the most common eye traumas. Blunt force tends to induce various degrees of damage to multiple eye tissues. In addition, the treatment efficacy is undesirable in clinical practice. Hence, the recovery of vision acuity is far from satisfactory. Recent investigations suggest that glutamic acid (Glu) acts as excitatory amino acid neu-

rotransmitters. The changes in the Glu level can reflect the pathological and physiological process of various diseases¹⁻², which are closely associated with the incidence of nerve-regression lesions and cerebral injuries. However, few studies focus on the relationship between Glu concentration and pathological lesions after retinal injuries have been conducted. Hence, we established rabbit models with high-speed bullet contusion as the primary injury factor in this investigation.

Materials and methods

Establishment of animal models

Twelve healthy two-month-old New Zealand rabbits (weighing 2 kg, regardless of sex) were selected from the experimental animal center of our hospital. They were in clean conditions with free access to water and food. Six rabbits were treated with left eye damages, and the other six animals were used as controls without any treatment. The experimental rabbits were subjected to intramuscular anesthesia using 25 mg/kg ketamine hydrochloride, and they were lying on their sides on an experimental table. The rabbits' eyelids were kept open using eye speculum, and TB bullets (weighing 0.201g on average) were shot at the central cornea approximately 1 cm from the rabbits' eyes at a speed of 90m/s. All the experimental models were established under same procedure.

Sample collection

Two experimental rabbits were sacrificed three hours, six hours, one day, three days, seven days, and 14 days after model establishment, respectively. A sclera puncture incision was made 3 mm from the corneascleral limbus using a puncture needle. A portion of 0.5 ml of vitreous was extracted from the incision using a No. 18 sterile syringe, and the vitre-

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ous was immediately stored at -80 degrees. The eyeballs were fixed in 4% formaldehyde.

Sample treatment

The vitreous samples were mixed with acetonitrile, centrifuged, and then $1\ \mu\text{l}$ of supernate was prepared for the detection of Glu and glutamine levels, using HPLC (HP Co., Ltd, United States) and Hypersil BDS 150×4.6 (Agilent Technologies, Inc. United States). Conventional HE staining was performed following the formaldehyde fixation.

Statistical analysis

Two independent sample *t*-tests were adopted. $P < 0.05$ was considered statistically significant.

Results

General eye changes

No ocular rupture was observed after bullet-shot treatment. Conjunctival edema and hyperemia became more apparent one day after the treatment. Viscous secretions were noted in the conjunctival sac. A moderate or severe degree of corneal opacity occurred, especially in the central corneal area. A blood clot was observed in the anterior chamber. Dyscoria, inert light response, and an unclear lens were also noted.

Three days following treatment, bulbar conjunctival edema, hyperemia, and eyelid swelling were relatively ameliorated. Viscous secretions from the conjunctival sac significantly decreased. Severe corneal haze occurred, especially in the central cornea. A slight amount of blood clot was noted within the anterior chamber. Dyscoria was noted, and other conditions were unclear.

Seven days after the treatment, bulbar conjunctival edema, hyperemia, and eyelid swelling were significantly improved. No abnormal secretions were noted. The corneal haze was worsened, especially in the central cornea. Dyscoria was indistinctly noted, while other conditions were unclear.

Fourteen days after the contusions, bulbar conjunctival edema, hyperemia, and eyelid swelling were basically recovered. A substantial amount of secretions was detected within the conjunctival sac. The corneal haze was slightly improved. Ulcer infiltration was noted in the central cornea. Neovascularization was observed at the peripheral cornea. A

blood clot was found in the anterior chamber, while other conditions were unclear.

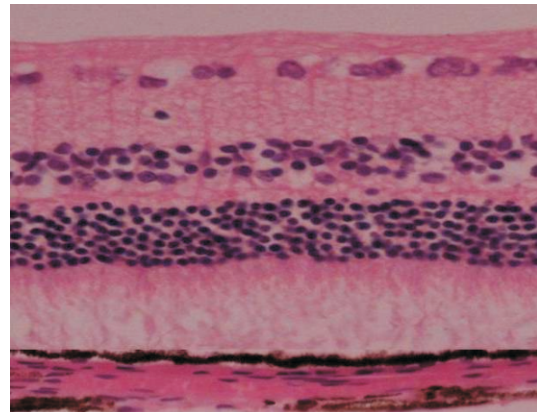


Figure 1 Normal retina (HE, $\times 100$)

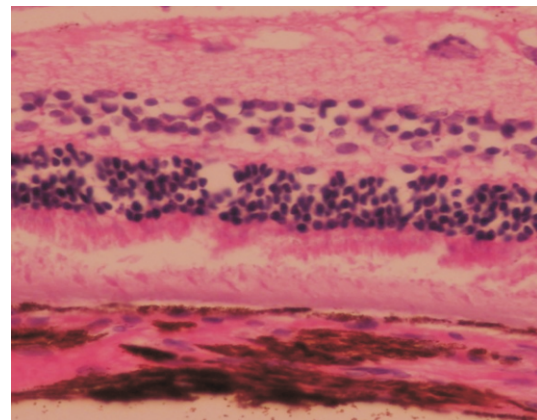


Figure 2 Injured retina, three hours after injuries (HE, $\times 100$)

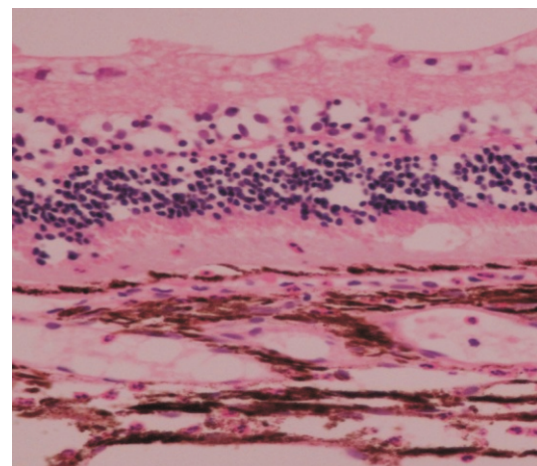


Figure 3 Injured retina, six hours after injuries (HE, $\times 100$)

Ocular changes under light microscope

In the control group, the eyes presented complete and distinct layer structures; the inner and outer segments of photoreceptor cells were distributed in order.

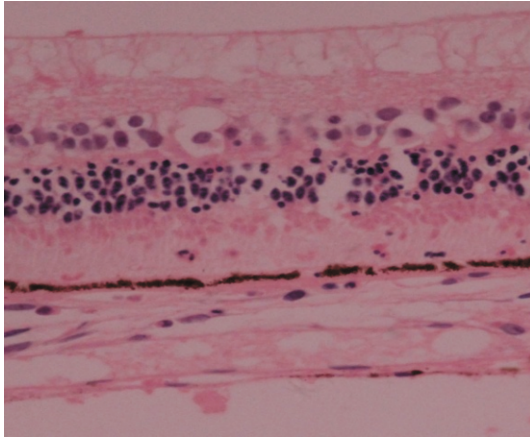


Figure 4 Injured retina, one day after injuries (HE, $\times 100$)

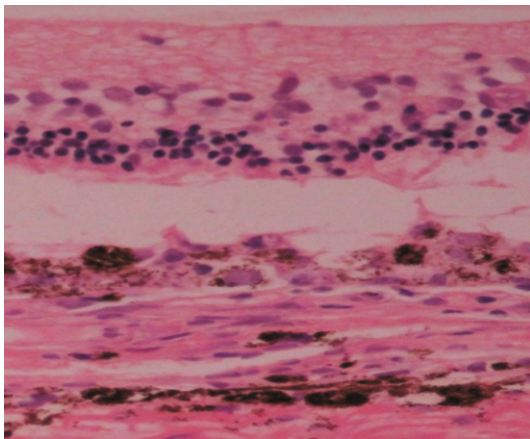


Figure 5 Injured retina, three days after injuries (HE, $\times 100$)

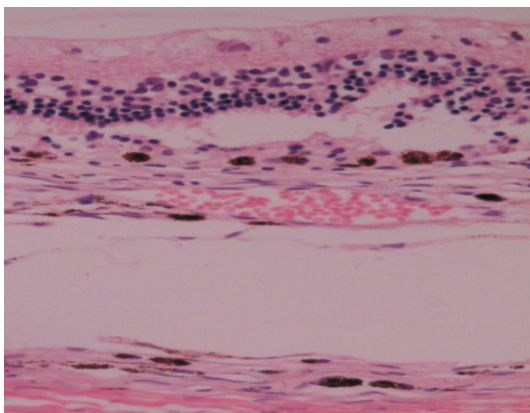


Figure 6 Injured retina, seven days after injuries(HE, $\times 100$)

Consecutive and regular pigments in the epithelial layer were observed under light microscopy, as shown in Figure 1.

Three hours after the injuries, the retinal tissue edema and intercellular edema, among the inner nuclear layer cells, were observed. The number of cells distributed in the ganglion cell layer became scarce.

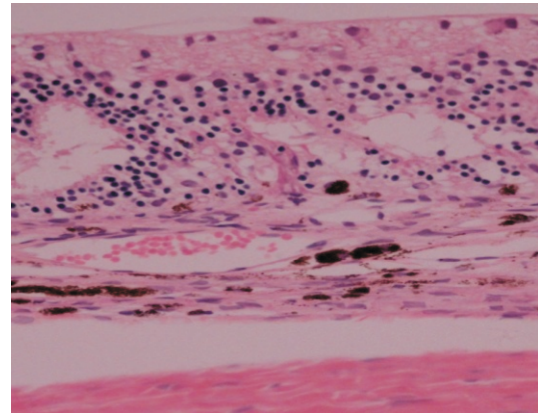


Figure 7 Injured retina, 14 days after injuries (HE, $\times 100$)

The internal limiting membrane was damaged. The inner segment of the photoreceptor layer showed a loose and disordered structure. Pigment epithelial-layer edema was noted. An exudative retinal detachment could be seen. Pigment cell degeneration was found in the choroid membrane.

Six hours after the injuries, the retinal tissue edema was aggravated; the continuity of the internal limiting membrane was disrupted. A relatively large amount of vacuolar degeneration cells were irregularly distributed in the inner nuclear layer. The outer nuclear layer cells were arranged in disorder. The outer segment of the photoreceptor layer presented abnormal structure, and the membrane disk fragments were distributed between the inner segment and the pigment epithelial cells. The continuity of the pigment epithelial layer was destroyed, and the partial cells were disrupted.

One day after the injuries, the amount of cells located in the ganglion cell layer and the inner and outer nuclear layers were significantly decreased. The inner segment of the photoreceptor layer cells was intact, whereas the outer segment was disrupted, shortened, and arranged irregularly. Limited cytolysis was found in the pigment epithelial layer, which was partially destroyed.

Three days after the injuries, the number of inner and outer nuclear cells was reduced. Normal plexiform structures disappeared. Both inner and outer segments of the photoreceptor layer cells were disrupted and disintegrated. The structure of the pigment epithelial layer was basically disrupted.

Seven days after the injuries, retinal edema was

further exacerbated. Both the inner and outer nuclear layers presented irregular structures. Small blood vessels in the choroid membrane were basically unobservable. A blood clot and a large amount of red blood cells had accumulated in vessels.

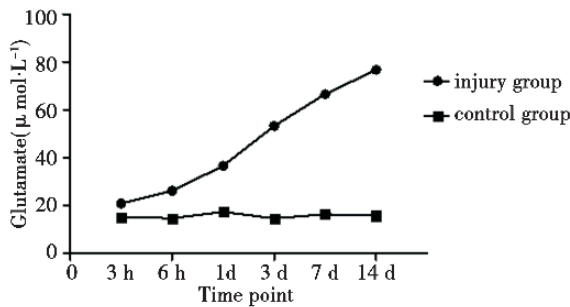


Figure 8 Value-time curves of the changes in vitreous Glu between the two groups

Fourteen days after the injuries, normal retinal tissues were destroyed. Choroidal membrane detachment occurred. The elasticity of the vascular wall of the choroidal membrane vessels had decreased.

Glu concentration in vitreous body

The level of Glu in the experimental rabbits was pronounced elevated, compared with that in normal controls (Figure 8), which presented extremely slight variations in terms of Glu content. A significant difference was noted between the two groups regarding Glu levels ($P=0.02$).

Discussion

High-speed object-induced eye contusion is prevalent during traffic accidents and fights. Severe dysfunction of vision acuity might occur even without the complication of eyeball perforation. In this study, we first utilized high-speed and low-quality objects to simulate eyeball contusions in rabbit models. The post-treatment pathological changes were much more serious than the experiments that used gravity and gas. We detected an evident increase in the Glu level of the vitreous body post-injuries, which was significantly higher than that reported by Huihua Chen, who used models with explosion injuries³. Hence, we paid more attention to the analysis of the relationship between excitatory amino acid and retinal damage.

The retina is extremely fragile and serves as one

of the essential tissues which form vision. Contusion-induced retinal lesions, a major reason for vision loss, possibly lead to the serious dysfunction of visual acuity. The results in this study revealed that retinal injuries were more severe than the anterior segment damages after a high-speed bullet was shot into the eyes. Evident retinal edema and exudation, pigment epithelial layer disruption, and choroidal vascular changes were observed. The number of inner and outer nuclear layer cells diminished greatly, and the inner and outer segments of photoreceptor layers were destroyed one day after the treatment. Normal retinal structures were mostly distorted 14 days after the injuries occurred. Both the inner and outer nuclear layer structures were displayed irregularly. The inner and outer segments of photoreceptor layer cells were disrupted and disintegrated. Choroidal detachment was noted. A blood clot was also found in the choroidal vessels. Severe retinal damages may be associated with mechanical characteristics when they are injured. When certain objects contact eyeballs at a high speed, the elastic eyeballs tend to deform and expand. The stress yielded during this process is reflected toward eyeball and orbital walls in all directions, and it likely produces an instant shearing force, which exacerbates the extent of damages, especially at back of the eyes.

Glu, prevalent in 90% of the retinal synapsis, serves as a major excitatory neurotransmitter for the central nervous system (CNS) of mammals. It mainly functions on the radial transmission pathway of photoreceptor cells, bipolar cells, and retinal ganglion cells (RGCs). Pathologically and excessively high levels of Glu likely cause damages and even kill neurons. To maintain the sensitive transmission property of the synapse, the excess Glu should be removed in a timely manner. The potential mechanisms of the excitatory toxic effect exerted by Glu include exudative damages that activate the Glutamate receptor (GluR), causing the inflow of Ca^{2+} , Na^+ , Cl^- , and H_2O . In the meantime, Na^+ and H_2O retention was noted within the cells. Extracellular fluid contains a high level of K^+ , with a low concentration of Na^+ , which further exacerbates neuron injuries. Second, the Ca^{2+} inflow induced delayed cellular injuries. An overload of Ca^{2+} causes excitatory amino

acid damages⁴. On one hand, excessive Ca^{2+} interferes with the respiratory function in the mitochondrial respiratory chain. On the other hand, it can activate the Ca^{2+} -dependent enzyme (NOS), phospholipase, endonuclease, and an intracellular signal transmission system (caspase family). Ca^{2+} , acting as a secondary messenger, is able to activate some chemicals related to cytotoxicity, such as NO. NO combines with O_2 and produces ONOO^- . First, such activity can cause cellular damages and even kill cells. Second, it enables tyrosine to generate 3-Nitrotyrosine (3-NT), which may cause tissue and cellular damages. NO is able to induce DNA injuries, block electron transfer in mitochondria by inactivating the Fe-S center, and, eventually, lead to the dysfunction of cell energy metabolism⁵.

Retinal damages weaken Müller cells' intake of Glu. Thus, glutaminase is released, accelerating the transformation from Gln to Glu. In addition, retinal cells were injured or dead post-trauma. On one hand, a high level of Glu within cytoplasm directly outflows. On the other hand, K^+ outflow enhances the discharge and promotes the release of Glu. Meanwhile, excessively high concentration of intracellular Glu enables neurons to further depolarize and then release Glu, forming a vicious cycle of positive feedback.

The vitreous body, as a content of the eyeball, supports and provides a nutrient supply to the retina.

Undoubtedly, the harmful substance contained in vitreous exerts toxic effects on retinal tissues. Vitreous body has no vessels. Most changes in the vitreous component and structure occur secondarily to lesions in the surrounding tissues. Vitreous possibly stores the metabolic products of the retina. Variations in Glu levels detected in the vitreous body potentially reflect the development of retinal injuries, because high levels of Glu induce excitatory toxic effects. However, the underlying mechanism remains elusive.

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