1. Introduction

The incidence rate of eye injury is 2%–10% in war. The traumatogenic factor of most eye injury in war is weapon or fragment of explosion. Eye rupture and penetrating injury are the most common in eye injuries in war. As a kind of severe open ocular trauma, eyeball rupture often combined with intraocular content loss and severe posterior pole retinal damage is called severe open ocular trauma characterized as poor prognosis and high blindness rate. The current therapeutic means cannot effectively improve cell death, cicatrization, and hyperblastosis and ganglion cell axon hyperplasia, etc.[1]. Lots of studies verified that apoptosis participates in the ischemia–reperfusion, light injury, retinal seperation and the photoreceptor cell injury of ocular contusion[2–4]. Ophthalmological studies mainly focus on hypertrophic vitreoretinopathy after injury[5–7], retinal response after injury (including retinal ganglion cell apoptosis[8–11], ganglion cell axon hyperplasia[12–14] and inflammatory reaction)[15,16], and retinal cell death and remodeling, etc[17,18].

p53, bcl–2 and Caspase–3 are important genes related to cell apoptosis. As an anti-oncogene, p53 has the function of transcription factor with its encoding protein in the nucleus. When cell is injured, wild-type p53 protein will timely initiate apoptosis in order to maintain the stability of the internal environment[18,19]. As an oncogene[20], with its encoding protein in the surface of mitochondrial inner membrane in cytolymph, bcl-2 can block the transmission of apoptotic signal, inhibit apoptosis and prolong the cell life. Caspase-3 is a known key molecule in the process of apoptosis, playing an important role in the execution of apoptosis. Some studies have shown that Caspase–3 activation is closely related to the apoptosis of pigment epithelial cell, outer nuclear layer cell and ganglion cell
caused by retinal injury[21–23]. In this study, eyeball rupture model was built. Then we observed the early clinical manifestation and tissue histopathological change of retina after rabbit eyeball rupture, the expression of p53, bcl-2 protein and Caspase-3 in retinal cells, the cell apoptosis of retinal cells and the spatial and temporal distribution of the swelling and necrosis retinal cells in order to provide experimental basis for the clinic treatment.

2. Materials and methods

2.1. Animal, reagents and instruments

After anesthesia, 48 healthy male New Zealand white rabbits, without eye diseases (turn out normal after the examination of outer eye and fundus), provided by the Experimental Animal Center in Henan Province, were selected in this experiment. Then they were randomly divided into control group and 1, 3, 6, 12 and 24 h after injury groups (experimental group), six groups in total and eight rabbits in each group. 0.4% eye drops (Santen Pharmaceutical Co., Ltd., Japan) and 3% pentobarbital sodium (Sigma Co., Ltd., USA) were prepared. Cornea confocal microscopy was from Japan NIDEK Company. Air gun was purchased from Chongqing Hongyuan Machinery Co., Ltd.

2.2. Establishment of the eyeball rupture model

This project was approved by the ethics committee of Zhengzhou University. Animal experiments conform to institutional standards. The care and use of animals was in accordance with institutional and national guidelines and in accordance with legal requirements in China. We adhered to the tenets of the Declaration of Helsinki or the NIH statement for the use of Animals in Research. All procedures were approved by the Institutional Animal Care and Use Committee and were carried out according to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The rabbits were anesthetized by intravenous injection of pentobarbital sodium from ear limbus (dose: 30 mg/kg×weight), and according to the degree of recovery, 1/5–1/4 of the initial dose was injected in order to sustain the anaesthesia[24]. After anaesthesia, the rabbit’s eyes were shot from the rabbit’s ear root along the line between ear root and center of the eyeball by an air gun, with the gunpoint 1 mm away the corneoscleral limbus, and the bullet was shot out of the nose root forming the anterior part eyeball rupture model. Then debridement and suturing was performed, and tobramycin was injected under conjunctiva. Penicillin was intramuscularly injected. 1, 3, 6, 12 and 24 h after injury, the rabbits was euthanized. Then the eyeball was immediately removed to detect the retinal morphological and functional changes.

2.3. Retinal electrophysiological observation

Electroretinogram (ERG) test was performed 1 h, 3 h, 6 h, 12 h and 24 h, respectively after injury[25]. Detection method: after full mydriasis and 30 min dark adaptation, SDY visual electrophysiological instrument was used to detect the change of ERG-b wave. The amplitude and peak value of ERG b wave were observed and detected.

2.4. p53, bcl-2 and Caspase-3 expression detected by immunohistochemical method and retinal morphological change

After enucleation of eyeball in the state of anesthesia at different time points, the surface blood in extracted eyeballs was washed by physiological saline. After cornea resection, the eyeballs were fixed for 24 h by 4% paraformaldehyde phosphate buffer. Then dehydration by alcoholic in gradient concentration, xylene transparent solution and paraffin embedding were performed. Four serial sections of posterior polar retina were resected with the 4 μm slice thickness. HE staining and MG–P–MY staining was respectively given to each two of them. Then the sections were put on the APES pretreated glass slide and put in the incubator overnight at 60 ℃. The steps of immunohistochemistry: (1) The dehydrated paraffin section was put into the 3% H2O2 solution for 10 min at room temperature, and then washed three times by distilled water; (2) The slide was soaked in the citric acid solution and heated to boiling by microwave oven. After 5 min–interval, this process was repeated twice; (3) The BSA confining liquid was added and set aside for 20 min at room temperature, and then the redundant solution was removed; (4) The rabbit anti–human antibody (or p53 antibody or Caspase–3 antibody) was added and overnight at 4 ℃. The sections were washed three times with 2 min for each time; (5) The biotinylated goat anti–rabbit IgG was added. The section was incubated in incubator for 20 min at 37 ℃. Then, the section was washed by PBS (pH=7.4) for three times with 5 min at a time; (6) SABC reagent was added. The section was incubated in incubator for 20 min at 37 ℃. Then, the section was washed by PBS (pH=7.4) for three times with 5 min at a time; (7) DAB coloration; (8) Slight restaining by hematoxylin, dehydration, transparency and mounting were conducted for microscope observation.

The distribution of positive RNA expression and apoptosis of retinal cells were detected by methyl green–pyronine–Matthew Huang (MG–P–MY) staining method[26,27]. Positive RNA cells (apoptotic cells) were pink or dark red stained under the observation of light microscope.
The following three kinds of cells can be seen under the ultramicroscope, normal cell with green dyed nuclear, pink apoptotic cell and swelling necrotic cells with reticular green dyed chromatin. The apoptotic index (AI) (AI= Number of positive RNA cells/total number of cells \times 100\%), number of oncosis cells and oncosis index (OI) (OI= Number of oncosis cells/total number of cells \times 100\%) were respectively calculated.

2.5. Statistical processing and data linear regression analysis

Statistical processing was performed by SPSS10.0 software. Firstly, test of homoscedasticity and normality was conducted. If heterogeneity of variance existed, change of variable was performed. After homogeneity of variance, the data difference of different time points in the same group was analyzed by Oneway–ANOVA. The least significant difference method was adopted to make the pairwise comparison between means. The relationship between two variables was analyzed by Spearman rank correlation analysis. Size of test: \( \alpha = 0.05 \). Regression analysis was performed for the correlation of data in each group by regress function in Matlab 7.0 software.

3. Results

3.1. ERG detection results

The ERG–b wave average amplitude of the eight rabbits in the control group was \((103.50 \pm 16.84)\) mv, while the latent time was \((97.30 \pm 6.58)\) ms. The ERG–b waves in the 1 h, 3 h, 6 h, 12 h and 24 h after injury group were evanescent waves (Figure 1).

![Electroretinogram](image1)

Figure 1. Electroretinogram.

3.2. Pathological test

Ordinary light microscope showed clear normal retinal ten–layer structure. 1 h after injury, fracture occurred in the retinal cone–rod cells, and there was no obvious change in the inner and outer nuclear layer and ganglion cells. There was slight hematocoele in the surface of retina; 3 h after injury, there was comparatively obvious fracture of cone–rod cells in the retinal cone–rod cells. Endochylema loose in inner nuclear layer and several vacuoles degeneration occurred. There was no obvious change in outer nuclear layer. The nuclear of ganglion cells was slightly stained. Obvious edema of nerve fiber layer can be observed. 6 h after injury, the fracture of cone–rod cells was obvious. Obvious vacuoles degeneration occurred in ganglion cells and inner nucleus layer. The number of ganglion cells began to decrease, and the layers of outer nuclear layer cell decreased. 12 h after injury, the number of ganglion cells and inner nuclear layer cells significantly decreased. The outer nuclear layer cells were arranged in disorder, and karyopyknosis can be observed. 24 h after injury, the retina structure was in disorder. The cone–rod layer was fractured and disappeared. Obvious vacuoles degeneration occurred in the inner and outer nucleus layer, and the number of which was significantly decreased. The karyolysis and pyknosis of the nuclear of ganglion cells continued (Figure 2).

![HE staining of retina after injury](image2)

Figure 2. HE staining of retina after injury.
A: normal retina \(\times 200\); B: retina of 1 h after injury \(\times 200\); C: retina of 3 h after injury \(\times 200\); D: retina of 6 h after injury \(\times 400\); E: retina of 12 h after injury \(\times 400\); F: retina of 24 h after injury \(\times 400\).

3.3. Results of MG–P–MY staining

No positive RNA expression (no apoptotic cell) was detected in retinal tissue of the 1 h group. There was few positive RNA expressions in the inner nucleus layer and ganglion cell layer in the 3 h group. In the 6 h group, the positive cells in inner nucleus layer increased, and few positive RNA expressions in outer nucleus layer appeared. In the 12 h group, obviously positive cells appeared. Large amount of RNA positive cells can be seen in the 24 h group (Figure 3). Compared the AI of control group with each experimental group, there was significant difference between the control group and the 1 h after injury group (\(P=0.033\)). However,
there was no significant difference between the control group and the other experimental groups ($P<0.01$) (Table 1).

The observation by ultramicroscope: No necrosis cells can be seen in the 1 h group. Necrosis cells appeared in 3 h reached the peak at 6 h and slightly decreased from 12 h.

**Table 1**

| Group          | AI     | OI     |
|----------------|--------|--------|
| Control        | 0.003 ±0.002 9 | 0.007 ±0.003 8 |
| 1 h after injury | 0.035 ±0.075 4 | 0.067 ±0.003 9 |
| 3 h after injury | 0.057 ±0.035 7 | 0.187 ±0.009 2 |
| 6 h after injury | 0.187 ±0.066 0 | 0.338 ±0.053 8 |
| 12 h after injury | 0.327 ±0.054 9 | 0.284 ±0.064 3 |
| 24 h after injury | 0.358 ±0.087 0 | 0.218 ±0.077 9 |

Comparison of apoptotic cell (RNA positive cell) with swelling and necrotic cell: No necrosis cells can be seen in the control group and 1 h group. Necrosis cells appeared in 3 h, reached the peak in 6 h and slightly decreased in 12 h.

From the aspect of cell percentage, within 6 h (including 6 h) after injury, the percentage of oncosis cells was larger than that of the apoptotic cells. 12 h (including 12 h) after injury, the percentage of apoptotic cells was larger than that of the oncosis cells. With prolongation of time, the number of oncosis and swelling cells showed the tendency of decline. However, the number of apoptotic cells tended to stabilization although slight augmentation can be seen 12 h after injury.

### 3.4. Expression of bcl–2, p53 and Caspase–3

bcl–2 protein was relatively low expression in the control and each experimental group, and there was no significant difference after homogeneity test for variance and analysis of variance after single factor multiple comparisons ($P>0.05$). Brownish yellow nucleus indicated that p53 was positively expressed. In this experiment, no brownish yellow staining can be seen in the normal retinal tissue, that is, p53 was not expressed. One hour after injury, seldom p53 was expressed in retina. 3 h after the injury, few p53 expressions can be found. p53 positive expression was obvious 6 h after injury and peaked in the 12 h. p53 mainly expressed in the inner nucleus layers and ganglion cell layer, and few expressed in the outer nucleus layer cell (Figure 4). bcl–2 and p53 protein expression and their correlation with cell apoptosis in the control group and each experimental group were demonstrated in Table 2, 3, 4. After homogeneity test for variance and single factor multiple comparison, there was significant difference between the control group and 3 h, 6 h, 12 h and 24 h group after the injury ($P<0.01$). Regression analysis of the relationship between p53 protein expression and cell apoptosis was performed by the regress function in Matlab 7.0 software using p53 protein expression as the independent variable $x$ and retinal cell apoptosis as the dependent variable $y$. The equation of linear regression: $y=0.926 \times x+1.572$ (Figure 5).

**Table 2**

| Group          | bcl–2    | p53     | Caspase–3 |
|----------------|----------|---------|-----------|
| Control        | 2.19±0.37 | 0.81±0.40 | 2.43±1.06 |
| 1 h after injury | 1.94±0.27 | 1.78±0.86 | 5.75±1.28 |
| 3 h after injury | 2.56±0.38 | 9.19±1.83 | 8.48±2.91 |
| 6 h after injury | 2.37±0.25 | 8.06±4.72 | 17.64±3.57 |
| 12 h after injury | 2.00±0.35 | 32.88±5.98 | 27.38±4.37 |
| 24 h after injury | 2.53±0.35 | 17.50±3.43 | 22.86±2.56 |

**Table 3**

| Group          | bcl–2($\bar{x}±s$) | Apoptosis($\bar{x}±s$) | Correlation coefficient ($r$) | $P$    |
|----------------|---------------------|------------------------|-----------------------------|--------|
| Control        | 2.19±0.37           | 0.44±0.32              | -0.109                      | 0.797  |
| 1 h after injury | 1.94±0.27           | 1.50±0.52              | -0.338                      | 0.412  |
| 3 h after injury | 2.56±0.38           | 3.06±1.15              | -0.679                      | 0.064  |
| 6 h after injury | 2.37±0.25           | 15.25±5.50             | 0.135                       | 0.749  |
| 12 h after injury | 2.00±0.35           | 28.25±7.21             | -0.404                      | 0.321  |
| 24 h after injury | 2.53±0.35           | 26.00±6.93             | 0.339                       | 0.412  |
4. Discussion

Compared with the control group in this experiment, there were significant differences in p53 expression and cell apoptosis levels. This observation suggests that p53 expression may play a crucial role in the regulation of cell apoptosis in the visual cells after injury.

Between Caspase-3 expression and cell apoptosis was performed by the regress function in Matlab 7.0 software (using Caspase-3 expression as the independent variable x and retinal cell apoptosis as the dependent variable y). The equation of linear regression: y=1.237x-5.024 (Figure 7).

Table 4
Correlation analysis between p53 and apoptosis.

| Group                   | p53 (x̄±s) | Apoptosis (x̄±s) | Correlation coefficient (r) | P  |
|------------------------|------------|------------------|-----------------------------|----|
| Control                | 0.81±0.40  | 0.44±0.32        | 0.884                       | 0.004 |
| 1 h after injury       | 1.78±0.86  | 1.50±0.52        | 0.902                       | 0.002 |
| 3 h after injury       | 9.19±1.83  | 3.06±1.15        | 0.741                       | 0.035 |
| 6 h after injury       | 8.06±4.72  | 15.25±5.50       | 0.827                       | 0.011 |
| 12 h after injury      | 32.88±5.98 | 28.25±7.21       | 0.965                       | 0.000 |
| 24 h after injury      | 17.50±3.43 | 26.00±6.93       | 0.847                       | 0.008 |

Table 5
Correlation analysis between Caspase-3 and apoptosis.

| Group                   | Caspase-3 (x̄±s) | Apoptosis (x̄±s) | Correlation coefficient (r) | P  |
|------------------------|------------------|------------------|-----------------------------|----|
| Control                | 2.43±1.06        | 0.44±0.32        | 0.903                       | 0.003 |
| 1 h after injury       | 5.75±1.28        | 1.50±0.52        | 0.894                       | 0.005 |
| 3 h after injury       | 8.48±2.91        | 3.06±1.15        | 0.857                       | 0.016 |
| 6 h after injury       | 17.64±3.57       | 15.25±5.50       | 0.925                       | 0.009 |
| 12 h after injury      | 27.38±3.37       | 28.25±7.21       | 0.971                       | 0.001 |
| 24 h after injury      | 22.86±2.56       | 26.00±6.93       | 0.887                       | 0.004 |

One hour after injury, the Caspase-3 expression in the ganglion cell layer and inner nucleus layers began to significantly increase, and continued this trend 3 h and 6 h after the injury, finally peaked at 12 h, but declined from 24 h. Compared with the control group, there was significant difference (P<0.01) (Table 2, Table 5, Figure 6). Caspase-3 positive cell mainly distributed in the inner nucleus layer and ganglion cell layer and few distributed in the outer nucleus layer cell. Regression analysis of the relationship

Figure 4. p53 expression in retinal cell by immunohistochemistry staining (×400).
A: p53 expression in control group; B: p53 expression 6 h after injury group; C: p53 expression 12 h after injury group; D: p53 expression 24 h after injury group.

Figure 5. Linear relationship between the p53 expression and cell apoptosis in visual cells after injury.

Figure 6. Caspase-3 expression in retinal cell by immunohistochemistry staining (×400).
A: Caspase-3 expression in control group; B: Caspase-3 expression 6 h after injury group; C: Caspase-3 expression 12 h after injury group; D: Caspase-3 expression 24 h after injury group.

Figure 7. Linear relationship between the Caspase-3 expression amount and cell apoptosis in visual cells after injury.
was no obvious change in the expression of bcl-2, and bcl-2 was relatively low expression both in the control group and experimental groups after injury. It seemed that bcl-2 protein cannot inhibit cell apoptosis. The reason for this may be that bcl-2 may participate in the retinal cell apoptosis after severe open ocular injury[28,29]. In terms of time, 12 h after injury, the peak of p53 and Caspase-3 expression appeared before the retinal cell apoptosis peak, existing a time difference in regulation, which implied that the p53 and Caspase-3 overexpression after rupture may lead to the cell apoptosis. Caspase-3 may play a key role in the early stage apoptosis of retinal cell. This experiment verified that apoptosis existed in retinal photoreceptor cells in the early stage of rabbit severe open ocular trauma, and early anti-apoptotic treatment may help to protect the residual retina and benefit the severe open ocular trauma.

Previous literatures verified that retinal cell apoptosis took place in the eye injury like ischemia–reperfusion, retinal detachment, and ocular blunt trauma, etc[30–32]. In our study, the main methods of retinal cell death after eyeball rupture were apoptosis and oncosis. From the aspect of cell percentage, within 6 h (including 6 h) after injury, the percentage of oncosis cells was larger than that of the apoptotic cells. 12 h (including 12 h) after injury, the percentage of apoptotic cells was larger than that of the oncosis cells. With prolongation of time, the number of oncosis cells showed the tendency of decline. However, the number of apoptotic cells tended to stabilization although slight augmentation can be seen 12 h after injury. Therefore, apoptosis and oncosis were the main ways of retinal cell death in this model, and especially apoptosis of retinal cell contributed more to retinal injury, which also indicated the researchers that the apoptosis of ganglion cells and inner nuclear layer cells may influence the prognosis and visual recovery in a great degree.

Eyeball rupture is frequently occurred in soldiers. In normal times or in times of war, the incidence of eyeball rupture (or other ocular trauma) ranked the first in the hospitalized officers and soldiers in the Ophthalmology Department, and eyeball rupture is also the main reason causing visual loss and influencing battle effectiveness. In this experiment, the shooting direction and distance were fixed, and the shooting direction was from back to front when building the eyeball rupture model. In this way, the possible injury to other organs and tissues was avoided. Compared with previous eyeball rupture models[33,34], this model is the most similar to the eyeball rupture in war wounds. This research will help to provide new idea and strategy to the early and timely treatment of eye injury in war or emergent events.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (81071723). I would like to express my warmest gratitude to Prof. Yu Zhu, my supervisor for instructive suggestions and valuable comments. Besides, I wish to thank teachers in Department of Ophthalmology, the First Affiliated Hospital of Zhengzhou University and Public Health College of Zhengzhou University. Finally, I greatly appreciate the teachers in Pathology Department of PLA 153 Central Hospital for their careful guidance and help in pathological specimen selection, pathological section preparation and dyeing process.

References

[1] Blanch RJ, Ahmed Z, Berry M, Scott RAH, Logan A. Animal models of retinal injury. Investig Ophthalmol & Visual Sci 2012; 53: 2913–2920.
[2] Hewish DR, Maden BE. Locations of large-scale features revealed by electron microscopy in relation to other features of the sequences. Biochem J 1973; 49: 56.
[3] Gelderblom D, Smit BJ, Bohm L. Effect of irradiation and endogenous nucleases on rat liver chromatin. Radiat Res 1984; 363–371.
[4] Batisatou A, Resneck WG, O’Neill A. Cytoplasmic components of acetylcholine receptor clusters of cultured rat myotubes: the 58kD protein. J Cell Biol 1991; 115(2): 435–446.
[5] Agrawal RN, He S, Spec E, Cui JZ, Ryan SJ, Hinton DR. In vitro models of proliferative vitreoretinopathy. Nat Protoc 2007; 2: 67–77.
[6] Westra I, Robbins SG, Wilson DJ. Time course of growth factor staining in a rabbit model of traumatic tracial retinal detachment. Graefes Arch Clin Exp Ophthalmol 1995; 233: 573–581.
[7] Saika S, Kono-Saika S, Tanaka T, Yamanaka O, Olnishi Y, Sato M, et al. Smad3 is required for dedifferentiation of retinal pigment epithelium following retinal detachment in mice. Lab Invest 2004; 84: 1245–1258.
[8] Fisher SK, Lewis GP, Linberg KA, Verardo MR. Cellular remodeling in mammalian retina: results from studies of experimental retinal detachment. Prog Retin Eye Res 2005; 24: 395–431.
[9] Fontainhas AM, Townes–Anderson E. RhoA inactivation prevents photoreceptor axon retraction in an vitro model of acute retinal detachment. Invest Ophthalmol Vis Sci 2011; 52: 579–587.
[10] Lewis GP, Chapin EA, Luna G, Linberg KA, Fisher SK. The fate of Muller’s glia following experimental retinal detachment: nuclear migration, cell division, and subretinal glial scar formation. Mol Vis 2010; 16: 1361–1372.
[11] Kayama M, Nakazawa T, Thanos A, Morizane Y, Murakami Y, Theodoropoulou S, et al. Heart shock protein 70(HSP70) is critical for the photoreceptor stress response after retinal detachment via modulating anti-apoptotic Akt kinase. Am J Pathol.2011; 178.
Optic nerve and vitreal inflammation are both RGC neuroprotective but only the latter is RGC axogenic. *Neurobiol Dis* 2010; 37: 441–454.

*Ahmed Z, Aslam M, Lorber B, Suggater EL, Berry M, Logan A. Optic nerve and vitreal inflammation are both RGC neuroprotective but only the latter is RGC axogenic. Neurobiol Dis* 2010; 37: 441–454.

*Silver J, Miller JH. Regeneration beyond the glial scar. Nat Rev Neurosci* 2004; 5: 146–156.

*Berry M, Ahmed Z, Lorber B, Douglas M, Logan A. Regeneration of axons in the visual system. Restor Neurosci* 2008; 26: 147–174.

*Benowitz LI, Yin Y. Optic nerve regeneration. Arch Ophthalmol* 2010; 128: 1059–1064.

*Trichonas G, Murakami Y, Thanos A, Morizane Y, Kayama M, Debouck CM, et al. Receptor interacting protein kinases mediate retinal detachment–induced photoreceptor necrosis and compensate for inhibition of apoptosis. Proc Natl Acad Sci USA* 2010; 107: 21695–21700.

*Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. Cancer Res* 1991; 51: 6304–6311.

*Weiner TA, Hartwell LH. The RAD 9 gene controls the cellcycle–responsive DNA damage in saccharomyce cerevisiae. Science* 1998; 241: 317–322.

*Baretton GB, Diebold J, Christoforis G. Apoptosis and immunohistochemical bcl–2 expression in colorectal adenomas and carcinomas: aspects of carcinogenesis and prognostic significance. Cancer* 1996; 77: 255–264.

*Jiang WJ, Niu YJ. Expression of Caspase–3 in retinal pigment epithelium after photic injury and effect of erythropoietin treatment. Chin Pharmacol Bull* 2012; 28(3): 443–444.

*Gao Y, Deng XG, Sun QN, He MF, Zhong ZQ. Temporal and spatial expressions of caspase–3, bax and bcl–xI in rat retina with MNU–induced photoreceptor damages. Chin J Ocul Fundus Dis* 2009; 25(2): 133–137.

*Wang YW. Surviving, cysteine protease–3 and interleukin–1 α in rats with retinal ischemia–reperfusion injury. Chin J Pract Med* 2010; 37(12): 40–41.

*Xu SY, Bian RL. Pharmacology experimental methodology. 3rd edition. Beijing: People’s Medical Publishing House; 2001, p. 190.

*Zhou J, Hui YN, Gao SY. Effect of vitrectomy on electroneurogram and ultrastructure of rabbits. Eye Res* 1996; 14: 92–94.

*Zhou JG, Liu CY, Huang YL, Liu JC, Gong ZJ. Method of MG–P–MY staining in detecting the neuronal apoptosis. J Navy Med* 2002; 23(4): 289–290.

*Ling QB. Practical technique for special pathological dyeing and histochemical assay. Guangzhou: Guangdong High Education Press; 1989, p. 194–197.

*Gou CF, Li L, Liu L, Zhang YM, Li XJ. Effect of coenzyme Q10 on expression of apoptosis–related genes in corneal epithelium of UV irradiation–induced injury rats. Chin J Ocular Trauma & Occupational Eye Dis* 2008; 30(8): 597–600.

*Nasrin A, Baharak A, Reza K. Concurrent cystic endometrial hyperplasia, ovarian luteoma and biliary cyst adenoma in an aged rabbit (Oryctolagus cuniculus): case report and literature review. Asian Pac J Trop Biomed 2012; 2(Suppl 2): S1975–S1978.

*Lam T T, Ahler A S, Tso MOM. Apoptosis and caspases after ischemia–reperfusion injury in rat retina. Invest Ophthalmol Vis Sci 1997; 39: 1193–1202.

*Hisatomi T, Skamoto T, Goto Y, Yamanaka I, Oshima Y, Hata Y, et al. Critical role of photoreceptor apoptosis in functional damage after retinal detachment. Curr Eye Res* 2002; 24(3): 161–172.

*An MX, Zhang XF, Zhang JS. Apoptosis of photoreceptor cell due to contusion of retinopathy. J Injuries & Occup Dis Eye* 2003; 25(5): 291–292.

*Lagerull JK, Jester JV, Smith RE. The effect of radial keratotomy on ocular integrity in an animal model. Arch Ophthalmol* 1982; 100(2): 319–320.

*Pinheiro MN Jr, Bryant MR, Tayyanipour R, Nassar–alla BA, Wee WR, McDonnell PJ. Corneal integrity after refractive surgery: Effects of radial keratotomy and mini–radial keratotomy. Ophthalmology* 1995; 102(2): 297–301.