· Original article ·

Experimental study on light injury of intense solar-stimulated exposure on rabbit retina

Yuan Gao[1], Xiu-Jun Peng[2], Bei Cui[1], Li-Qun Cao[1], Li-Wei Qin[1], Xia Zhang[2]

[1] Physical Examination Center for Recruitment of Navy Pilots, Navy General Hospital, Beijing 100048, China; [2]Department of Ophthalmology, Navy General Hospital, Beijing 100048, China

Correspondence to: Xiu-Jun Peng. Department of Ophthalmology, Navy General Hospital, Beijing 100037, China. Pxj@vip. sina. com

Received: 2012-12-17 Accepted: 2013-02-15

模拟强日光照射导致兔眼视网膜光损伤的实验研究

高 原[1],彭秀军[2],崔 蓓[1],曹利群[1],秦力维[1],张 霞[2]
(作者单位:[1]100048 中国北京市,海军总医院海空勤体检中心;[2]100048 中国北京市,海军总医院眼科)
作者简介:高原,副主任医师,海军总医院海空勤体检中心主任,医学硕士。主要研究方向:眼底病及各种屈光不正的手术矫治。
通讯作者:彭秀军,主任医师,海军总医院眼科主任,医学博士。主要研究方向:眼底病及白内障手术. Pxj@vip. sina. com

摘要

目的:探讨短时间模拟强日光照射对兔视网膜组织结构的影响及光损伤的致伤机制。

方法:健康新西兰白兔25只随机分为5组,30000lux模拟日光照射兔眼15min和30min。照射后不同时间取视网膜进行光镜、透射电镜观察并对视网膜感光细胞凋亡率进行检测。

结果:15min光照组2d时观察:光感受器细胞外节盘膜结构紊乱,板层结构离散,内节线粒体肿胀,部分嵴断裂;外颗粒层细胞胞浆有空泡样改变;感光细胞凋亡率为3.26%±0.98%。30min光照组2d时观察,外节盘膜结构紊乱,板层结构重度离散,内节线粒体肿胀,嵴断裂;外颗粒层细胞胞浆有空泡样改变;感光细胞凋亡率为3.63%±1.25%。30min光照组7d时观察,外节盘膜组织结构紊乱,板层结构消失,空泡化,内节线粒体肿胀,嵴断裂;外颗粒层细胞胞浆肿胀,大量空泡样改变,排列紊乱,部分胞膜破坏;感光细胞凋亡率为19.63%±1.32%。30min光照组14d时观察,外节盘膜组织结构恢复较规则,板层结构较致密,内节线粒体肿胀,嵴断裂;外颗粒层细胞胞浆肿胀减轻,排列较整齐;感光细胞凋亡率为18.98%±1.13%。

结论:30000lux模拟强日光照射15min可导致兔视网膜急性光损伤,模拟强日光照射可导致视网膜感光细胞发生退行性变性,感光细胞凋亡是损伤发生的重要机制。

关键词:光损伤;视网膜;感光细胞;形态学;凋亡

引用:高原,彭秀军,崔蓓,等. 模拟强日光照射导致兔眼视网膜光损伤的实验研究. 国际眼科杂志 2013;13(3):429-433

Abstract

• AIM: To investigate the effect of retinal organizational structure on short term intense light exposure rabbit eye model and the mechanism of light injury.

• METHODS: Totally 25 healthy New Zealand white rabbits were randomly divided into 5 groups. Rabbit eyes were exposed to 30000 lux simulated solar for 15 minutes and 30 minutes. Then the retinal tissue ultrastructure was observed and photoreceptor cells apoptosis at different time after exposure was detected.

• RESULTS: Examination results of 15 minutes light exposure group at 2nd day: membrane structure of photoreceptor outer segment was disordered; lamellar structure was discrete; mitochondrial of the inner segment were swollen and some ridges were ruptured; cell plasm of external granular layer was vacuolated-like; apoptosis rate of photoreceptor cells was 3.26%±0.98%. Examination results of 30 minutes light exposure group at 2nd day: membrane structure of outer segment was disordered; lamellar structure was severely dispersed; mitochondrias of the inner segment were swollen and ridges were ruptured; cell plasm of external granular layer was vacuolated-like; apoptosis rate of photoreceptor cells was 3.63%±1.25%. Examination results of 30 minutes light exposure group at 7th day: organizational structure of outer segment membrane was disordered; lamellar structure was disappeared and vacuolated; mitochondrial of the inner segment were swollen and ridges were ruptured; cell plasm of external granular layer was swollen, vacuolated largely, and disordered; partial membranes were damaged; apoptosis rate of photoreceptor cells was 19.63%±1.32%. Examination results of 30 minutes light exposure group at 14th d: organizational structure of outer segment membrane was recovered orderly; lamellar structure was more compact; mitochondrial of the inner segment were swollen and ridges were ruptured; swollen cell plasm of external granular layer was mitigatory and arranged more regularly; apoptosis rate of photoreceptor cells was 18.98%±1.13%.

• CONCLUSION: Results demonstrate that 30000lux stimulated solar radiation for 15 minutes led to acute photodamage of rabbit retina, showing retinal photoreceptor cells degeneration which is the important mechanism of photoreceptor cells apoptosis.

• KEYWORDS: light injury; retinal; photoreceptor cells; morphology; apoptosis

DOI:10.3980/j. issn. 1672-5123. 2013. 03. 03

Citation: Gao Y, Peng XJ, Cui B, Cao LQ, Qin LW, Zhang X. Experimental study on light injury of intense solar-stimulated exposure on rabbit retina. *Guoji Yanke Zazhi*(*Int Eye Sci*) 2013;13(3):429-433

INTRODUCTION

Light is a driving force of visual evolution and development. Retina is an important tissue perceiving external light which normal function is critical to the formation of visual images. But light can result in retinal photodamage when light intensity or duration exceeds the retina endurance[1-2]. The word "light pollution" have been well known[3]. Refracting media in eyes has a strong focusing effect and can converges incident beam into a tiny optical spot, which renders light intensity reaching the retina per unit area is 10^5 times of that reaching the corneal. In this experiment, light injury was caused by light from xenon lamp close to spectrum of sunlight, 30000lux, stimulated daylight of cloudy day, filtered out infrared light, which did not increased temperature significantly and reduced the heat damage. Experimental conditions were close to sunlight causing retinal damage. Rabbit eyes were illuminated by 30000lux in this study, then retina photoreceptor cells pathologic change were observed dynamically after light irradiation using light microscope, transmission electron microscopy and TUNEL(terminal deoxynucleotibyl transferase mediated dUTP-biotin nick end labeling) technology and explore the effect of intense solar exposure on organizational structure of the retina and light injury mechanism.

MATERIALS AND METHODS

Animals Totally 25 healthy adult rabbits without sex limiting weighing between 2kg and 2.5kg were used in all experiments. All experimental protocols were conducted to the Guide for the Care and Use of Laboratory Animals prepared by the National Institues of Health and approved by the Ethics Committee for Animals Experiment of the Navy general hospital. Animals were randomly divided into 5 groups that is the control groups including 5 rabbits, 15 minutes irradiated groups 5 rabbits and 30 minutes groups 15 rabbits(30 minutes light exposure group at 2 day, 30 minutes light exposure group at 7th day, 30 minutes light exposure group at 14th day, 5 rabbits in each group). The animals were housed in a temperature-controlled 21-24℃ and humidity 40%-60% with a 12-hour light/dark cycle for 7 days. All animals used dark adaptation 24h before intense light irradiation.

Light injury model building According to the literature[4], the pupils of rabbit were dilated by compound tropicamide to 8mm. Fixed rabbits head and eye speculum which is suffered light in order to let eyes exposed to xenon light which have 3000lux light intensity and filter out infrared light. The irradiation time was controlled in 15 minutes and 30 minutes according to group. The rabbits were returned to rear with normal conditions after 30 minutes of dark adaptation. Then the changes of 15 minutes light exposure group after 2 days, and 30 minutes light exposure group after 2, 7, and 14 days were observed. The unexposed group was reared normally after mydriasis as the control and specimens were separated after 2 days for examination.

Morphological Examination

Optical microscope examination Eyeballs of experimental animals were enucleated under general anesthesia, removed anterior segment, and fixed in 10% Formalin solution for 48 hours. Specimens were obtained at lmm from the optic nerve root temporal side in the sagittal plane, paraffin-embedded, prepared 5μm serial sections, stained routinely by HE and labelled by TUNEL. The further observation was taken by microscope[5].

Transmissionelectron microscopy(TEM) examination Eyeballs of experimental animals were enucleated under general anesthesia, removed anterior segment, and fixed in a 2% formaldehydum polymerisatum 2.5% glutaraldehyde solution at 4℃ for 2 hours. Retina of papilledema superior temporal area was cut into rectangle tissue mass(1mm×0.5mm), then washed 3 times using dimethyl arsenic sodium buffer(pH 7.2), fixed in 1% osmium acid at 4℃, dehydrated successively and embedded directionally. Observe morphology using TEM by double stained with uranyl acetate and lead citrate after ultrathin section[6].

Detection of retinal apoptosis TUNEL staining was performed on paraffin-embedded section by using the *in situ* cell death detection kit. To compare the TUNEL-positive cells in each group, the TUNEL-positive cells in the retinal ganglion cells (RGC) layer of each sample were counted in ten high power fields(HPF, 400×). Three section per eye were averaged to calculate the apoptosis rate of photoreceptor cells with average values.

Statistical Analysis For all analyses, the individual performing the analysis was blinded to sample condition. For the analysis of cell count results, a non-parametric Kruskal-Wallis ANOVA was used followed by Dunn's test. Statistical significance was declared if a $P<0.05$.

RESULTS

Optical Microscope Examination There were no obvious pathological changes in normal retinal tissue which displayed integral and clear structure (Figure 1). Figure 1D showed representative samples of HE staining from the 30 minutes light exposure group at 7th day. Compared with the control groups, it can clearly be seen the structure ruptured and cytoplasm vacuolization between the rod and the cone of retina, the cell of external granular layer loosed and some of the cell nucleus disappeared, the cell of internal granular layer was in a condition of confusion and disordered, most of the nucleus in ganglionic layer occasionally vanished and the cytoplasm vacuolated. The same examination results of 15 minutes light exposure group at 2nd day (Figure 1B) is minor compared with Figure 1D and that of 30 minutes light exposure group at 2nd day (Figure 1C) was relatively light but heavier than that of 15 minutes in 2 days. Meanwhile the structure was in repair status of 30 minutes light exposure group at 14th day, the tissue structure obviously recovered, the cells order neatly arranged and other changes more little (Figure 1E).

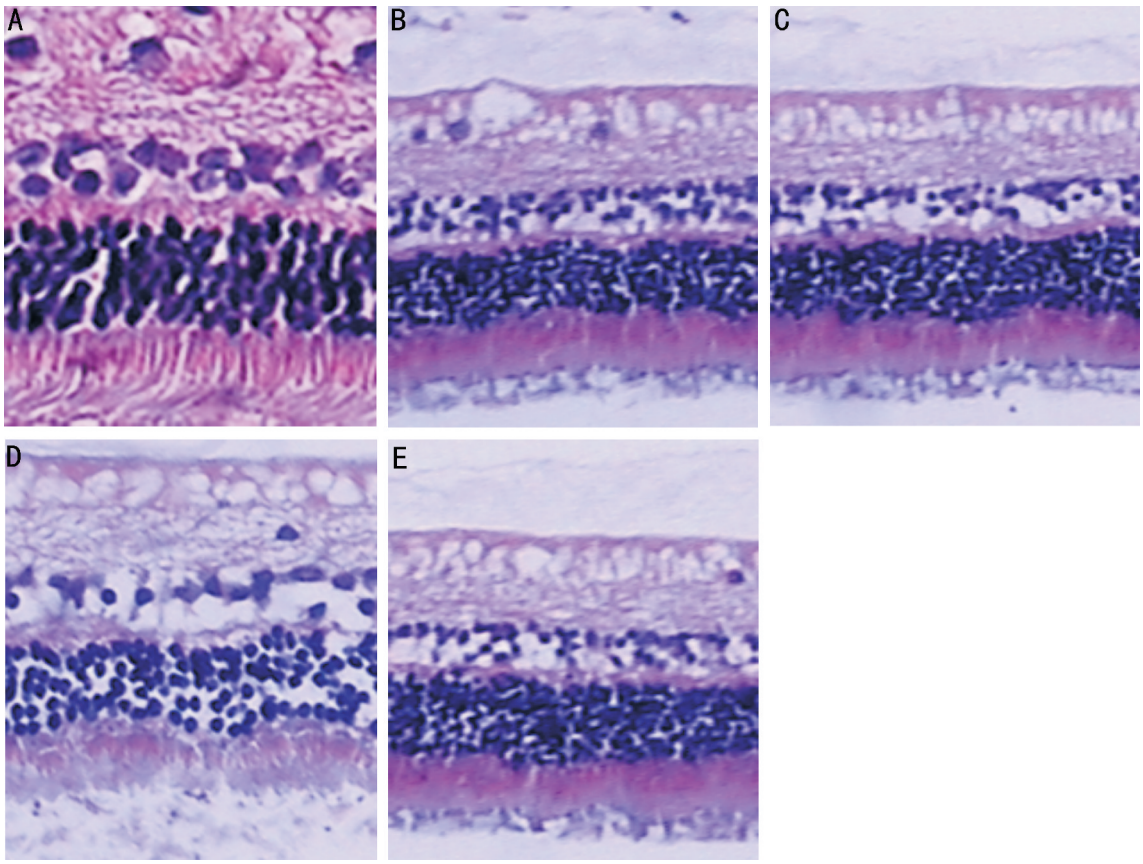

Figure 1 Optical microscope examination of retinal tissue morphology (HE×100) A: Control Group; B: 15 minutes exposure at 2nd day; C: 30 minutes light exposure group at 2nd day; D: 30 minutes light exposure group at 7th day; E: 30 minutes light exposure group at 14th day.

TEM Examination Retinal structures of rabbits in control group were almost normal. Examination results of 15 minutes light exposure group at 2nd day: membrane structure of outer segment between the rod and the cone was disordered; the lamellar structure was discrete; mitochondrial of the inner segment was swollen and individual ridges were ruptured (Figure 2A); cell plasm of external granular layer was vacuolated-like; cell plasm of internal granular layer was swollen mildly (Figure 2F); cell morphology of ganglionic layer was almost normal. Examination results of 30 minutes light exposure group at 2nd day: membrane structure of outer segment between the rod and the cone was disordered and dispersed severely; mitochondrias of the inner segment were swollen and ridges were ruptured; cell plasm of external granular layer was vacuolated-like; cell plasm of internal granular layer was swollen mildly; cell plasm of ganglionic layer was swollen mildly. Examination results of 30 minutes light exposure group at 7th day: membrane structure of outer segment between the rod and the cone was disordered, and lamellar structure disappeared and was vacuolated (Figure 2B); mitochondrias of the inner segment were swollen and ridges were ruptured; cell plasm of external granular layer was swollen; the cell plasm which contained a lot of vacuoles was disordered; partial membranes were damaged (Figure 2D); cell plasm of internal granular layer was highly swollen and changing to vacuolated-like; partial membranes were damaged; cell plasm in ganglionic layer was swollen and membrane was damaged. Examination results of 30 minutes light exposure group at 14th day: membrane structure of outer segment between the rod and the cone are regularly arranged, and lamellar structure was more compact (Figure 2C); mitochondrias of the inner segment were swollen and ridges were ruptured; swollen cell plasm of external granular layer was mitigatory and arranged more regularly (Figure 2E); the cell plasm of internal granular layer was less swollen and arranged more orderly; cell plasm of ganglionic layer was lightly swollen.

TUNEL Staining The TUNEL method has become the most widely used in situ test for the study of apoptosis, which is based on the specific binding of TdT to 3'-OH ends of DNA. Normal retinal TUNEL staining was negative. In light exposure groups, TUNEL assay illustrated that several positive cells were located in the nucles, which show yellow or brown granular staining and some of nucleus was stained heavily at the edge and lightly in centre, referring to a ring appearance (Figure 3B). The TUNEL-positive cells/HPF of 3.26% ± 0.98% cells in 15 minutes light exposure group at 2nd day, 3.63% ±1.25% cells in 30 minutes light exposure group at 2nd day, 19.63% ±1.32% cells in 30 minutes light exposure group at 7th day ($P<0.01$ *vs* control group) and 18.98% ± 1.13% cells in 30 minutes light exposure group at 14th day ($P<0.01$ *vs* control group) (Figure 4).

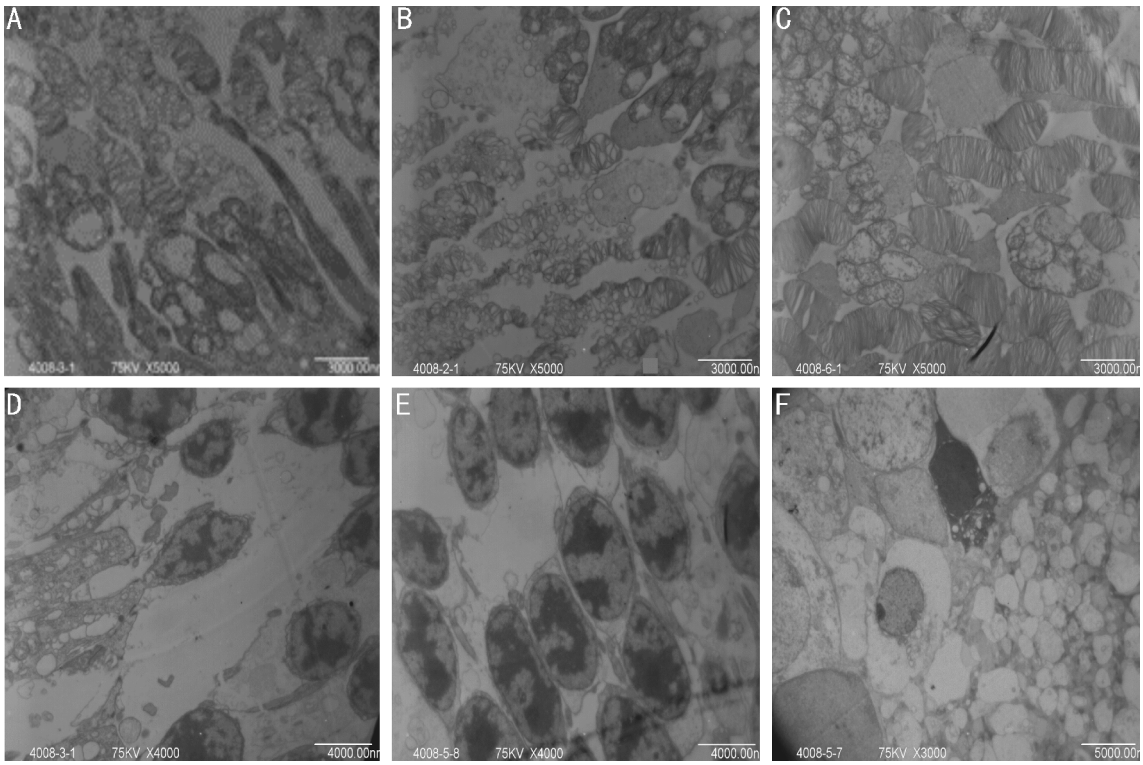

Figure 2 TEM examination of retinal tissue morphology A: 15 minutes light exposure group at 2nd day, the membrane structure of outer segment was disorder; lamellar structure was discrete; mitochondrial of the inner segment was swollen; ridges were broken (×5000); B: 30 minutes light exposure group at 7th day, the membrane structure of outer segment was disorder; lamellar structure disappeared and was vacuolated; mitochondrias of the inner segment were swollen; ridges were ruptured (×5000); C: 30 minutes light exposure group at 14th day, the membrane structure of outer segment regularly arranged; lamellar structure was more compact, mitochondrias of the inner segment were swollen; ridges were cracked (×5000); D: 30 minutes light exposure group at 7th day, the cell plasm of external granular layer was swollen, sparse, disordered and largely calculated; partial membranes were damaged (×4000); E: 30 minutes light exposure group at 14th day, the swollen cell plasma of external granular layer was mitigatory and arranged more regularly (×4000); F: 15 minutes light exposure group at 2nd day: cell plasm of internal granular layer was swollen; nucleus was occasionally observed (×3000).

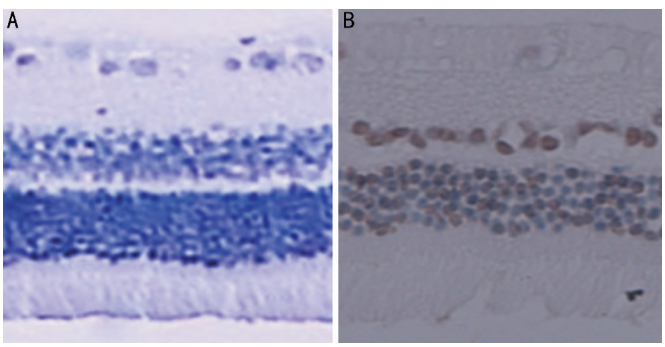

Figure 3 Detection of retinal apoptosis A: Control group; B: 30 minutes light exposure group at 7th day.

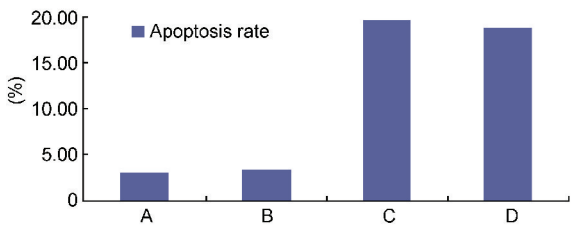

Figure 4 Apoptosis rate in each group A: 15min group at 2nd day; B,C,D: 30min group at 2nd,7th, 14th day, respectively.

Apoptosis analysis of 30 minutes light exposure group at 7th day In external granular layer of the retina, dispersed positive cells can be seen, which show granular yellow or brown. The positive cells were located in the nucleus. Partial nucleus was stained heavily on the brink and lightly in centre, referring to a ring appearance (HE×200). Note a significant increase ($P<0.01$) as compared to control group.

DISCUSSION

The retina is an important structure of visualsystem which is also vulnerable to light injury[7]. Eye can converge the incident beam into a tiny optical spot owing to the focusing effect of the refractive medium, that renders light intensity reaching the retina per unit area for 10^5 times of that reaching the corneal. It has been reported the visual impairment caused by sunlight in the early 17th century by Swiss doctor Bonetus[8]. The sunlight even below the thermal damage threshold can cause experiment mouse retinal damage[9]. Retina prones to suffer from photodamage when there is absence of special protection under strong sunlight (intensity to be 130000lux), especially in the high mountains, deserts, islands and the ship deck and other environments which will

enhance the light radiation effect.

In this study we adopt xenon lamp which spectrum is similar to sunlight as experiment source that is better than previous experiment conditions with low dosage visible light such as fluorescent lamp, operating microscope and lasting time[10,11]. Our experiment results indicate that this light about 30000lux simulating daylight of cloudy day can filter out infrared light and not cause a significant temperature rise wherefore can reduce the heat damage and approach the conditions caused by sunlight.

Ultrastructure has been shown to have photodamage on rabbit eye under the conditions simulating sunlight irradiation 15 minutes, 2 days (intensity 30000lux). The optical microscope examination results demonstrated the photoreceptor cells represented damaged appearance at 2 days after light injury and the severity was more serious at 7th day. Then tissue was shown repair performance at 14th day. The changes of outer segment of membranous disc were more obvious compared with all structural changes.

The research of pathologic changes of the retina photodamage are more focused on the early post-injury[12-13]. And also found the ultrastructural changes of retinal damage by electron microscope[14]. Our experiment performed dynamic observation of the long time that shows retinal structural damage experienced from continued increase non-acute to gradually repair. And the form of damage expressed as selective degeneration of retinal photoreceptor cells. If the majority of the lost photoreceptor cells in this process occurs necrosis, it would induce significant inflammation and following local tissue scarring. But histomorphometric results didn't show these phenomena. In our experiment, the apoptosis of retinal cells was accompanied by the injury of the retinal structure that proposed the retinal cells after light injury arose degenerative changes through apoptosis, during which the cell was broken into several parts containing nuclear debris and apoptotic bodies coated by membrane. Then these apoptotic bodies were eventually swallowed by phagocytic cells. In this process, there was no inflammatory response [15-16] as the cell contents doesn't leak. The apoptosis cells we observed are only a small portion of the actual apoptosis population owing to the cells apoptosis non-synchronized and the individual cells apoptosis transient process though the apoptosis rate is not very high in our experiment. Therefore, the apoptosis might be an important mechanism of photoreceptor cell death after light injury and also a major role during the retinal external granular layer degeneration.

REFERENCES

1 Kaldi I, Berta A. Progesterone administration fails to protect albino male rats against photostress-induced retinal degeneration. *Eur J Ophthalmol* 2004;14(4): 306

2 Ranchon I, Chen S, Alvarez K. Systemic administration of phenyl-N-tert-butylnitrone protects the retina from light damage. *Invest Ophthalmol Vis Sci* 2001;42(6): 1375

3 Zhang WH, Huang XT, Tang RH. Effect of MDA and SOD on retinal ultraviolet light damage in mice. *Int J Ophthalmol* 2010;10(8):1490-1492

4 Cheng YY, Sang AM, Lu H. The establishment of animal models for retinal injury. *Int Rev Ophthalmol* 2010;34(3):149-151

5 Wang P, Peng Q, Gao Z, Gao Y, Wang XG, Zhao J. the protective effect of alpha-lipoic acid on retinal light damage in mice. *J Med Res* 2011;40(4):98-100

6 Peng H, Ma SH, Ma S, Dong Y, Wang WQ. Effect of compound light capsule on rats' retinal light injury induced by operation microscope, an electron micrescope observation. *J Kunming Med Univ* 2011;(11):31-35

7 Guo YQ, Yang PL, Guo YZ. The study of retinal light damage. *Strait J Sci* 2005;(5):68-69

8 Duke Elder S. Nonmechanical Injuries. *St. Louis C. V. Mo sby* 1972;837

9 Noell WK, Walker VS, Kang BS, Berman S. Retinal damage by light in rats. *Invest Ophthalmol Vis Sci* 1966;5(5):450-473

10 Zhao YP, Sun SY, Niu JJ. Progression of prevention and treatment of retinal light injury. *Int J Ophthalmol* 2009;9(11):2132-2135

11 Li GL, Zhu XH. Dynamic observation on retinal photochemical injury in rats. *Shanxi Med J* 2000;29(3): 204-206

12 Organisciak DT, Li M, Darrow RM. Photoreceptor cell damage by light in young Royal College of Surgeons rats. *Curr Eye Res* 1999;19(2):188-196

13 Organisciak DT, Darrow RM, Barsalou L, Krishnan Kutty R, Wiggert B. Susceptibility to retinal light damage in transgenic rats with rhodopsin mutations. *Invest Ophthalmol Vis Sci* 2003; 44(2):486-492

14 Wang X, Hu SX, Li W. Role of Caspase-3 in acute light damage to retina of rats. *Chin Med Sci* 2007;22(1):44-48

15 Medema JP. Apoptosis: Life and death in a flash. *Nature* 1999;29(398):756-757

16 Jacobson MD, Weil MM, Raff MC. Programmed cell death in animal development. *Cell* 1997;88(3):347-354