WITHDRAWN: Role of Short-wavelength Blue Light in the Formation of Cataract and Expression of Caspase-1, -11 and Gasdermin D in rat Lens Epithelium Cells: Insight into the Novel Pathogenesis of Cataract.

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The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Background

Cataracts have been verified to be associated with a number of risk factors. The sun and artificial light sources, including light-emitting diode (LED) and fluorescent light tubes, are the primary sources of short-wavelength blue light. With the increasing popularity of blue-rich LED-backlit display devices, our eyes are now exposed to more short-wavelength blue light than they were in the past. The goal of this study was to evaluate the role of short-wavelength blue light in the formation of cataract. Additionally, the pathogenesis of cataracts after short-wavelength light exposure was investigated.

Methods

SD rats were randomly divided into 2 main groups: a control group (10 rats each for the 4-, 8-, and 12-week groups) and an experimental group (10 rats each for the 4-, 8-, and 12-week groups). The rats in the experimental group were exposed to a short-wavelength blue LED lamp for 12 hours per day. After exposure to the blue LED lamp, the rats were maintained in total darkness for 12 hours, after which a 12-hour light/dark cycle was resumed. The intensity of the lamp was 3000 lux. At the end of the short-wavelength blue LED lamp exposure (for 4, 8, and 12 weeks), the expression levels of caspase-1, caspase-11 and gasdermin D (GSDMD) in rat epithelium cells (LECs) were examined in rat epithelial cells (LECs) using qRT-PCR and Western blotting analyses.

Results

After 6 weeks, cataracts had developed in the experimental rats (4/20 eyes). The clarity of the lens then gradually worsened with the duration of exposure. Twelve weeks later, all of the rat eyes had developed cataracts. Then the expression levels of caspase-1, caspase-11 and GSDMD at 4, 8, and 12 weeks were significantly higher in samples from rats exposed to a short-wavelength blue LED lamp than samples from control rat ($p < 0.05$).

Conclusion

The data indicate that pyroptosis play a key role of in cataracts induced by short-wavelength blue light exposure, highlighting caspase-1, caspase-11 and GSDMD as possible therapeutic targets for cataract treatment. This study might provide new insight into the novel pathogenesis of cataracts.

Background

Globally, cataracts are the leading cause of blindness and accounting for nearly half (47.8%) of all blindness cases [1]. In a population-based survey of 9 provinces in mainland China, blindness (prevalence of 1.66%) was caused by cataracts in approximately half of patients aged 50 years and older even though the rate of unoperated cataract was reduced from 2006 to 2014[2]. In a study of populations aged 50 years and older in rural northern China, approximately 28.6% of participants had poor visual

outcomes, and cost was the most common barrier (73.9%) to cataract removal [3]. To date, the only therapeutic method for cataracts is surgery, which has the potential for serious postoperative complications, e.g., increased intraocular pressure (IOP) and corneal edema. Hence, the studies of cataractogenesis are vital for developing effective therapeutic modalities for the prevention and treatment of cataracts.

It is certified that cataracts are associated with a number of risk factors e.g., drugs, malnutrition, aging, exposure to ultraviolet (UV) light, and diabetes mellitus [4–6]. Blue light is short-wavelength electromagnetic radiation (400–500 nm) and carries the highest amount of energy. Blue light has attracted increasing attention because short-wavelength blue light has the potential to induce damage to the retina [7,8]. The sun and artificial light sources, including light-emitting diodes (LEDs) and fluorescent light tubes, are the primary sources of short-wavelength blue light. With the increasing popularity of blue-rich LED-backlight display devices, such as mobile smartphones, tablets, and computers, our eyes are now exposed to more short-wavelength blue light now than they were in the past [9]. A link between short-wavelength blue light exposure and the formation of cataracts has been suggested, but the evidence is inconclusive.

Pyroptosis is a novel inflammatory form of programmed cell death. Caspases are a family of aspartate-specific cysteine proteases with 15 mammalian members. The majority of caspases can be grouped into apoptotic caspases and inflammatory caspases [10]. The former group includes caspases –2, –3, –6, –7, –8, –9 and–10, and the latter group consists of caspases–1, –4, –5 and- 11[11]. Apoptosis and pyroptosis rely on specific caspases to induce their respective programmed cell death pathways[12]. Inflammatory caspases (caspases–1, 4, –5 and –11) induce a form of necrotic programmed cell death, namely, pyroptosis[13]. This type of cell death can be triggered by the canonical and noncanonical inflammasome signaling pathways[14–16]. The canonical inflammasome pathway is activated by caspase–1 and is assembled by cytoplasmic sensors, such as NOD-like receptors (NLRs). The noncanonical inflammasome pathway is related to caspases–4 and –5 in humans and caspase–11 in mice and is stimulated by immune system activators, such as lipopolysaccharide (LPS) [17].

The activity of caspase–1 can result in the maturation of IL–1β and IL–18 and the cleavage of gasdermin D (GSDMD) to induce pore opening and pyroptosis[18]. In contrast to canonical inflammasomes, the noncanonical inflammasome seems to be composed solely of pro-caspase–11, which plays the role of the sensor as well as the executor [19,20]. The morphological changes that occur during pyroptosis include plasma membrane fracture, water influx, cellular swelling, osmotic lysis, and proinflammatory cellular content release [21]. Accumulating evidence confirms that pyroptosis is involved in the nosogenesis of noninfectious and infectious diseases [22,23].

In the present study, we hypothesized that pyroptosis is involved in the mechanism associated with the occurrence and development of cataract. A rat model of short-wavelength blue light exposure was established, and relative changes in pyroptosis factors in rat lens epithelial cells (LECs) were analyzed.

Materials and methods

Animals

Six-week-old male Sprague-Dawley (SD) rats (weighing 210 ± 30 g) were provided by the Central Laboratory of the First Affiliated Hospital of Harbin Medical University, China. The housing conditions were as follows: room temperature (18–25°C), 75% humidity, 10 rats per cage, standard rat chow and access to water ad libitum. This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University, China.

SD rats were randomly divided into 2 groups: a control group (10 rats each for the 4-, 8-, and 12-week groups) and an experimental group (10 rats each for the 4-, 8-, and 12-week groups). The rats in the control group were exposed to continuous indoor natural light from 6 AM to 6 PM and then were maintained on a daily routine of total darkness (12 hours indoor natural light/dark cycle). The light exposure experiment of the rat model was carried out in Harbin, China (44°04'–46°40'N) from July 2018 until September 2018. In Harbin, summer solstice occurs on the 21st of June when the day length is the longest in the year. The intensity of the indoor natural light was measured daily. The average intensity of indoor natural light showed a maximum of 2100 lux around noon.

The rats in the experimental group were exposed to a short-wavelength blue LED lamp (455–460 nm) (Grass Farmer's Home Co., Ltd., Shenzheng, China) for 12 hours per day. After exposure to the blue LED lamp, the rats were maintained in total darkness for 12 hours, after which a 12-hour light/dark cycle was resumed. To improve the directional uniformity of the radiation and avoid punctate sources, metallic boxes containing rows of blue LED light with a diffuser were placed above 3 metallic cages, leaving 1 m space for air circulation and temperature maintenance at 18–25°C. The light intensity was controllable. The intensity of the lamp was checked at the position of rats' eyes everyday by a digital light meter. Each cage was placed in a ventilated white environment, and the distribution of light in the cage was homogenous regardless of the rat position. To our knowledge, the relationship between the intensity of blue light and the damage to the lenses of unrestrained rats is unknown. An illuminance of 1000–3000 lux was selected to explore the roles of light on retinal damage in previous studies [24–26]. We adopted the illuminance intensity to 3000 lux to study the potential influence of blue LED light on rat lenses. All rats in both groups were treated with compound tropicamide eye drops and atropine gel to achieve mydriasis.

Lens clarity changes in all rats were examined as described previously[27]. At the end of short-wavelength blue LED lamp exposure (4, 8, and 12 weeks), the rats were subjected to treatment according to the methods described [27]. A mixture of ketamine (60 mg/1 kg) and xylazine (7.5 mg/1 kg) was intraperitoneally injected. The rats were sacrificed by cervical dislocation under anaesthesia. The absence of sounds of breathing and heartbeat, namely cardiac and respiratory arrest, through a stethoscope were confirmed the rats were euthanized in this study. Both eyes were rapidly enucleated and the intact lenses

were removed after the rats were confirmed dead. The lens capsules were frozen in liquid nitrogen. The lens capsules from both eyes were used for one experiment regardless of the cataract grade.

Quantitative real-time PCR (qRT-PCR)

qRT-PCR was used to examine the relative expression of caspase–1,–11, and GSDMD in all control and experimental rat lens epithelial cell samples as previously described [27].

Western blotting analysis

Western blotting was used to quantify tissue protein expression as previously described [27]. Detailed methods and table are provided in the supplementary materials.

Statistical analyses

Statistical analyses were performed using SPSS 23.0 software (SPSS Inc., USA). Data were given as the mean ± standard error (SE). Differences between groups were analyzed by two-way ANOVA. $p < 0.05$ was considered significant.

Results

Effects of short-wavelength blue light on cataract formation

Each rat in both the experimental and control groups was examined biweekly by slit-lamp microscopy to detect changes in lens clarity changes. The lenses of the rats in the control group appeared transparent throughout the 12-week observation period. At 4 weeks after short-wavelength blue LED lamp exposure, all rat lenses in both the control and experimental groups were transparent (Fig. 1). However, after 6 weeks, cataracts had developed in the experimental rats (4/20 eyes), as indicated by equatorial and postcortical vacuoles (grade 2). After 8 weeks of exposure, 25% (5/20 eyes) of the rat eyes displayed grade 2 cataracts, and 25% (5/20 eyes) displayed grade 3 cataracts (Fig. 2). Twelve weeks later, 100% of the rat eyes exposure to blue light exhibited cataracts. Of all the eyes with cataracts, 55% (11/20 eyes) of the rat eyes displayed grade 2 cataracts, 25% (5/20 eyes) displayed grade 3 cataracts, and 20% (4/20 eyes) displayed mature cataracts (grade 4) (Fig. 3).

Expression of caspase-1

To determine whether caspase-1 was associated with the changes in rat eyes after exposure to a short-wavelength blue LED lamp, we examined the relative expression of caspase-1 in all control and experimental rat lens epithelial cell samples using qRT-PCR. The relative expression levels of caspase-1 were significantly higher in samples from short-wavelength blue LED lamp-exposed rats at 4, 8, and 12 weeks than in those from control rats at the corresponding time points (p 0.01) (Fig. 4). The mean log10 ($2^{\Delta\Delta CT}$) of expression of caspase-1 in the rat samples was 1.27, 5.30, and 12.79 after 4, 8, and 12 weeks of exposure, respectively, showing that the caspase-1 levels were 4.17-fold higher in the samples from the

8-week blue light exposure group than in the samples from the 4-week blue light exposure group and 10.07-fold higher in the samples from the 12-week blue light exposure group than in those from the 4-week blue light exposure group. However, there was no significant difference in caspase-1 expression among the control rats at 4 weeks, 8 weeks and 12 weeks in the blue light exposure group ($p > 0.05$).

Western blot analysis revealed that caspase-1 protein expression was upregulated in the experimental group (4, 8 and 12 weeks after short-wavelength blue LED lamp exposure) compared to that in the control group at the same time points ($p < 0.05$) (Fig. 5). The present results indicate that short-wavelength blue LED lamp exposure is associated with increased expression of caspase-1 in rat LECs.

Expression of caspase-11

We investigated the mRNA expression levels of caspase-11. Caspase-11 mRNA expression was significantly higher in rats exposed to short-wavelength blue light (4-, 8-, and 12-week blue light exposure groups) than in healthy control rats ($p< 0.05$). Caspase-11 expression levels in the 8-week and 12-week blue light exposure group samples were increased by 2.40-fold and 5.61-fold, respectively, compared to those in the 4-week blue light exposure group ($p < 0.05$) (Fig. 6). These results revealed that short-wavelength blue light exposure could induce pyroptosis in rat lens cells in a time-dependent manner.

As shown in Fig. 7, compared with the control group, the experimental groups exhibited markedly increased protein expression of cleaved caspase-11 in rat LECs at 4, 8 and 12 weeks. Furthermore, the cleaved caspase-11 levels were also higher in rat lens cells after 12 weeks of short-wavelength blue light exposure than in rat lens cells after 4 and 8 weeks of exposure ($p<0.05$). These results revealed that short-wavelength blue light exposure could induce pyroptosis in rat LECs in a time-dependent manner.

Expression of GSDMD

The GSDMD activity in rat lens cells subjected to short-wavelength blue light exposure was also measured in this study (Fig. 8) using qRT-PCR. GSDMD expression was significantly increased after 4, 8 and 12 weeks of short-wavelength blue light exposure, and longer exposure times at the same intensity had more dramatic effects on the increase in GSDMD expression ($p<0.05$).

In the present study, Western blot analysis using an anti-GSDMD antibody was performed to investigate the change in GSDMD levels in rat LECs after 4, 8, and 12 weeks of short-wavelength blue light exposure. Cleaved GSDMD levels increased after short-wavelength blue light exposure, and as the exposure time increased, cleaved GSDMD expression increased (Fig.9). The relative expression level of cleaved GSDMD in the samples from the 12-week-exposed rats was 1.50-fold and 1.17-fold higher than that in the samples from the 4-week- and 8-week-exposed rats, respectively ($p<0.05$).

Discussion

Due to their considerable advantages, such as high energy savings, low power consumption, and high light efficiency characteristics, LEDs will gradually replace traditional incandescent light sources.

Additionally, the potential biological photochemical damage to the retina caused by LED light has also raised public concern [28]. Previous studies have shown that blue light (400–500 nm) induced oxidative stress and cellular damage in retinal tissues[29,30]. Conversely, laboratory results demonstrated that reducing blue light (430 nm) transmission through a blue-light filter by 50% could reduce approximately 80% of photochemical damage to the retina[31]. In agreement with these findings, blue light-filtering intraocular lenses have been proposed as a protective measure against the blue light damage to the retina [32].

The human crystalline lens is continuously exposed to solar and artificial light throughout its lifetime. It acts as an “optical windshield,” which is exposed to not only longer wavelength ultraviolet (UV) radiation (300–400 nm) but also the full range of visible light (400–760 nm). When visible light passes through the cornea into the eye, it first comes in contact with the human lens epithelial cells (hLECs), which are the active metabolic part of the lens. Studies have confirmed that cumulative visible light exposure may accelerate the development of cataract [33].

The normal construction and function of LECs are crucial for maintaining transparency and stabilizing the intracellular environment of the entire lens. The dysfunction of the lens epithelium may lead to edema in superficial cortical lens fibers that undergo degeneration and produce a localized zone of vacuolization, and subsequently, mature cataract [34].

An increasing number of studies have focused on learning the mechanisms of pyroptosis in different diseases. In the present study, we report that after 6 weeks of short-wavelength blue LED lamp exposure, cataracts had developed in the experimental rats. In addition, pyroptosis markers, including caspase–1, caspase–11, and GSDMD, were investigated. The present study demonstrated that the expression levels of caspase–1, caspase–11 and GSDMD were significantly increased in rat LECs after 4, 8, and 12 weeks of exposure to a short-wavelength blue LED lamp. The results confirmed that pyroptosis may play a vital role in the formation of cataract after short-wavelength blue light exposure.

Our present research demonstrated that cataracts had developed in the experimental rats after 6 weeks of blue light exposure, as indicated by equatorial and postcortical vacuoles. The clarity of the lens then gradually worsened with the duration of short-wavelength blue light exposure. After twelve weeks of blue light exposure later, 20% (4/20 eyes) displayed mature cataracts. The process of cataract formation determined by this study is consistent with that determined by an earlier study [34]. In the present study, the phenotype is cataract involving the lens fiber cells too.

A previous study demonstrated that pyroptosis participates in the oxidation of human LECs and may be involved in the initiation and progression of noncongenital cataracts. Caspase–1 plays an important role in the process of pyroptosis in H_2O_2-treated lens epithelial cells during the formation of cataract, and the caspase–1 and IL–1β pathways may be involved in this pathological process [35]. However, the role of short-wavelength blue light in the formation of cataract and the relative expression of pyroptosis markers, such as caspase–1, caspase–11, and GSDMD, in vivo is still unknown. In the present study, we

demonstrated that short-wavelength blue light-induced activation of caspase–1, caspase–11, and GSDMD triggered cataracts in a pyroptotic manner.

Caspase–1, which is a crucial marker in the process of pyroptosis [36], is activated by the NLRP3 inflammasome. Caspase–1 mediates proinflammatory programmed cell death in response to exogenous and endogenous stimuli to protect cells. Caspase–1 dysfunction is closely associated with different diseases [37,38]. The results of the current study show that caspase–1 expression was increased in short-wavelength blue light-exposed rat lens cells in a dose-dependent manner.

Most previous studies have focused on targeting the canonical inflammasome pathway. However, emerging studies have actively explored the regulatory role of the caspase–11 noncanonical inflammasome in noninfectious diseases. Studies have indicated that aging activates the NLRP1 inflammasome, resulting in the processing of caspase–1 and the upregulation of caspase–11 [39]. Assembly and activation of the NLRP1 inflammasome involves caspase–1 and caspase–11 activation, which subsequently leads to the maturation and secretion of IL–1β and IL–18 [40,41]. In the present study, the qRT-PCR and Western blotting analysis results showed increased expression of caspase–11 in rat LECs after short-wavelength blue light exposure. We hypothesize that caspase–11 might be activated by naturally occurring intracellular molecules in inflammatory conditions and that these intracellular inflammatory molecules might bind directly to caspase–11, subsequently activating caspase–11 noncanonical inflammasomes and leading to the pathogenesis of cataract after short-wavelength blue light exposure. Further investigation is required to confirm this hypothesis. The authors plan to explore the association between the NLRP1 inflammasome and pyroptosis by caspase–11/ IL–1β and IL–18 signaling in the LECs after the short-wave length blue light exposure.

Studies revealed that GSDMD is activated by caspases –1, –4, –5, and –11, all of which split GSDMD into an N-terminal effector domain and a C-terminal inhibitory domain [20,42]. We found that the mRNA expression levels of GSDMD were increased, suggesting that pyroptosis was activated by short-wavelength blue light. Morphological evidence of gasdermin-mediated pore formation and membrane rupture in LEC pyroptosis also needs to be further proven.

Our study has potential limitations. Simply evidencing increased levels of caspases and GSDMD in SD rats, which were nocturnal, and in the albino strain, does not thoroughly explain the association between short-wavelength blue light exposure and the formation of cataract in humans. Inhibitors of caspase–1 and caspase–11 in hLECs exposed to short-wavelength blue light need to be employed to investigate pathways involved in the pyroptosis process. Second, the intensity and duration of the blue light used in the study were not physiologically relevant, and additional studies on the safety of long-term exposure to low levels of blue light are needed to determine the effects of blue light on the eye. Finally, although the increased level of caspase–1 may suggest that pyroptosis is involved in this process, it is difficult to determine whether other cell death types, such as apoptosis and necrosis, are involved in cataract formation in lens cells concurrently under short-wave length blue light. Therefore, further research is needed to address this problem.

Conclusions

We demonstrated that expression of the pyroptosis factors caspase–1, caspase–11, and GSDMD in rat LECs was increased after short-wavelength blue light exposure. Thus, therapeutic strategies that aim to prevent LEC pyroptosis may inhibit the expression of related pyroptotic factors and may be beneficial for treating age-related cataracts.

Abbreviations

LED: light-emitting diode LECs Lens epithelial cells; IOP: intraocular pressure; UV: ultraviolet light; GSDMD: gasdermin D; NLRs: NOD-like receptors; LPS: lipopolysaccharide; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; PBS: Phosphate-buffered saline; hLECs: human lens epithelial cells.

Declarations

Acknowledgments

Not Applicable

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Availability of data and materials

I had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Author's Contributions

YW, MZ, YS, and XW performed the in vivo experiments. ZS, HL, and KL analyzed the data. ZL designed the research and wrote the main manuscript and all authors reviewed and commented on the manuscript.

Ethics approval and consent to participate

All animal protocols were approved by the First Affiliated Hospital of Harbin Medical University, Harbin, China.

Consent for publication

Not available.

Competing interests

The authors declare that they have no competing interests.

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Figures

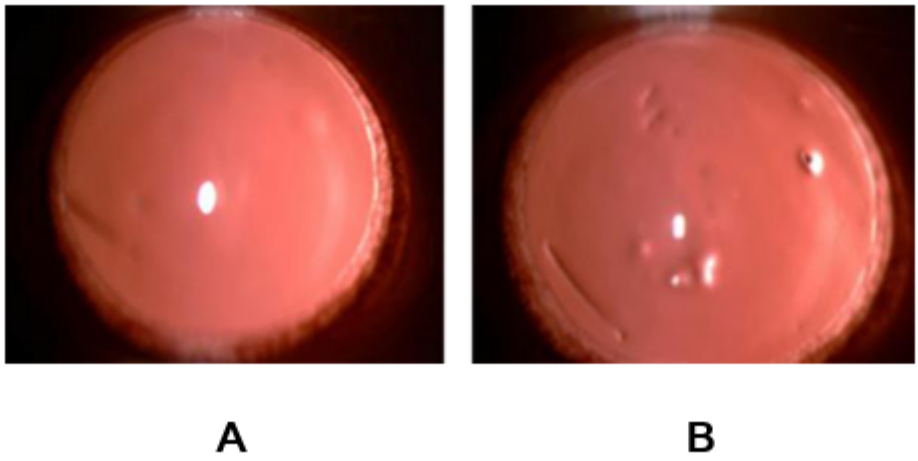


Figure 1

The slit-lamp observation images of rat lenses in the experimental group and control group after 4 weeks A: control group: the rat lenses were all transparent; B: experimental group: the rat lenses were all transparent.

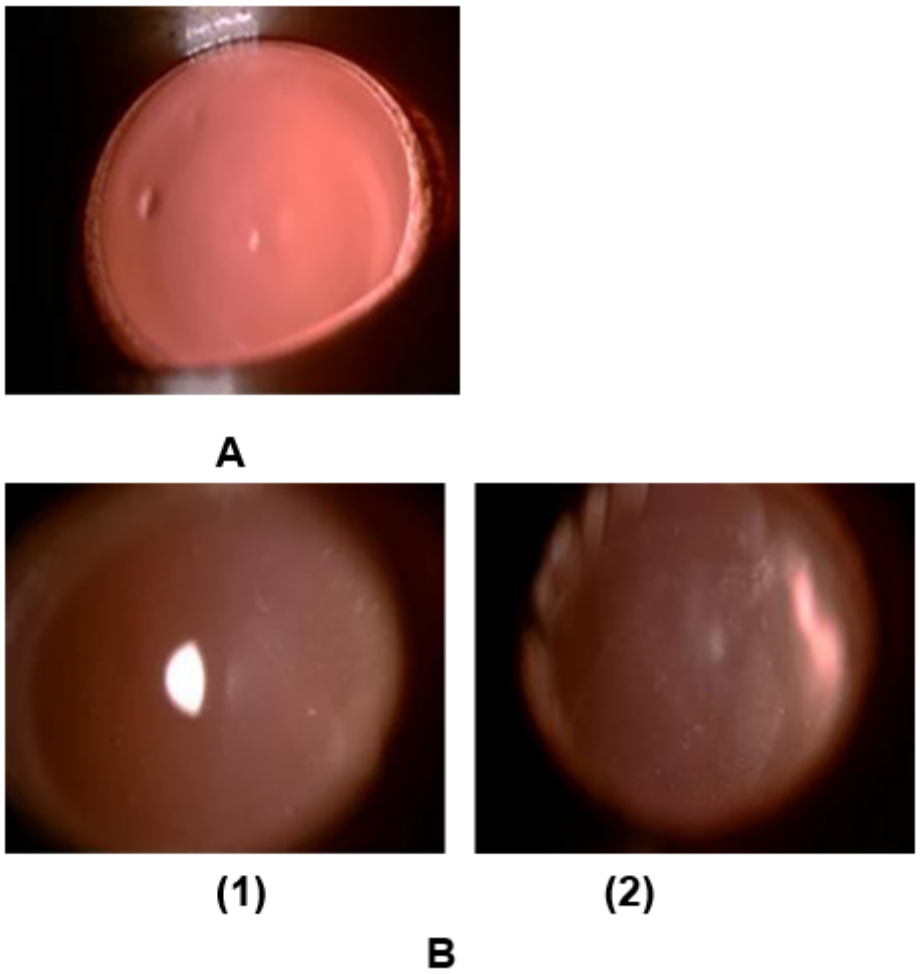


Figure 2

The slit-lamp observation images of rat lenses in the experimental group and control group after 8 weeks A: Control group: the rat lenses were all transparent; B: experimental group: (1) Grade 2 cataracts. (2) Grade 3 cataracts.

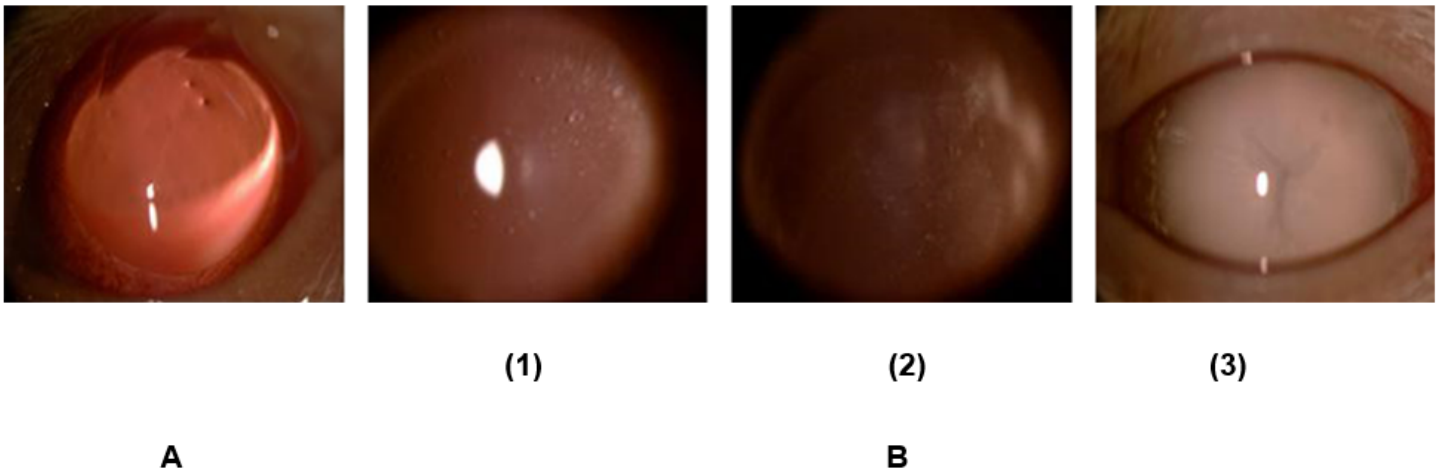


Figure 3

The slit-lamp observation images of rat lenses in the experimental group and control group after 12 weeks A: Control group: the rat lenses were all transparent; B: experimental group: (1) Grade 2 cataract, (2) Grade 3 cataract, and (3) Grade 4 cataract.

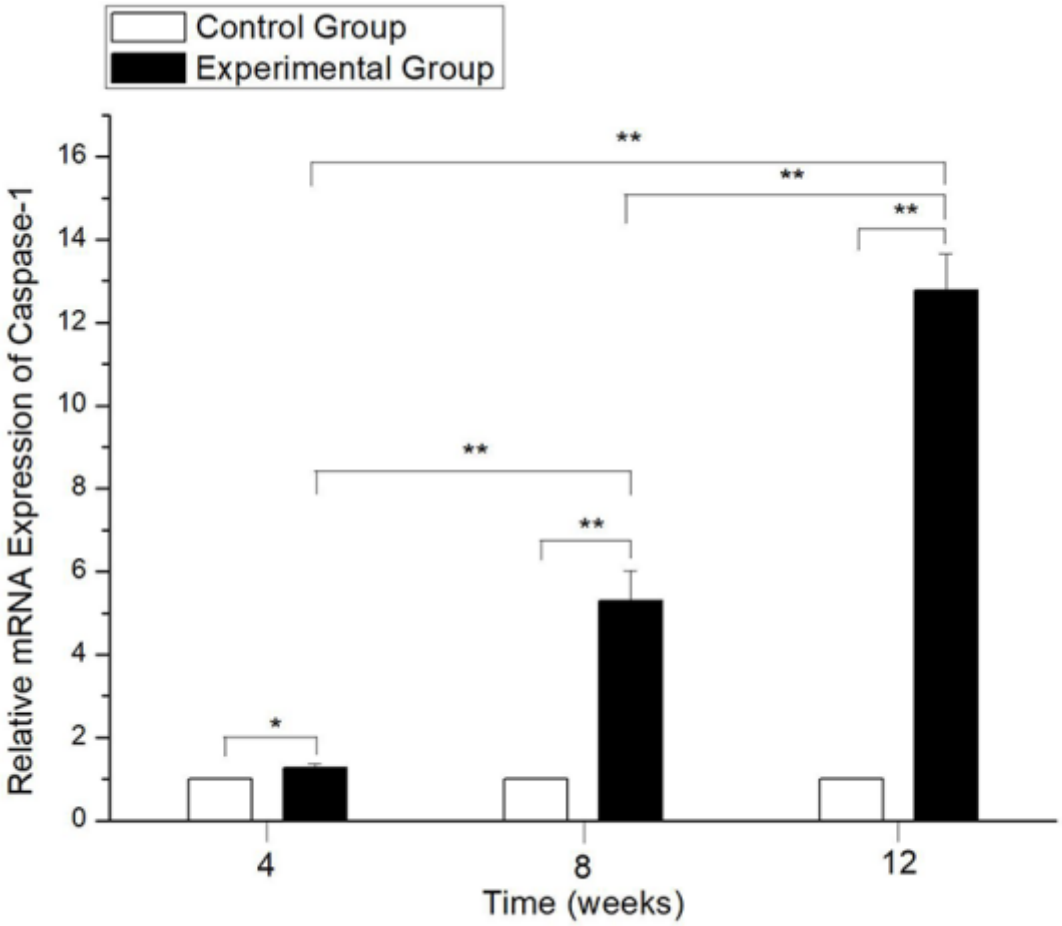
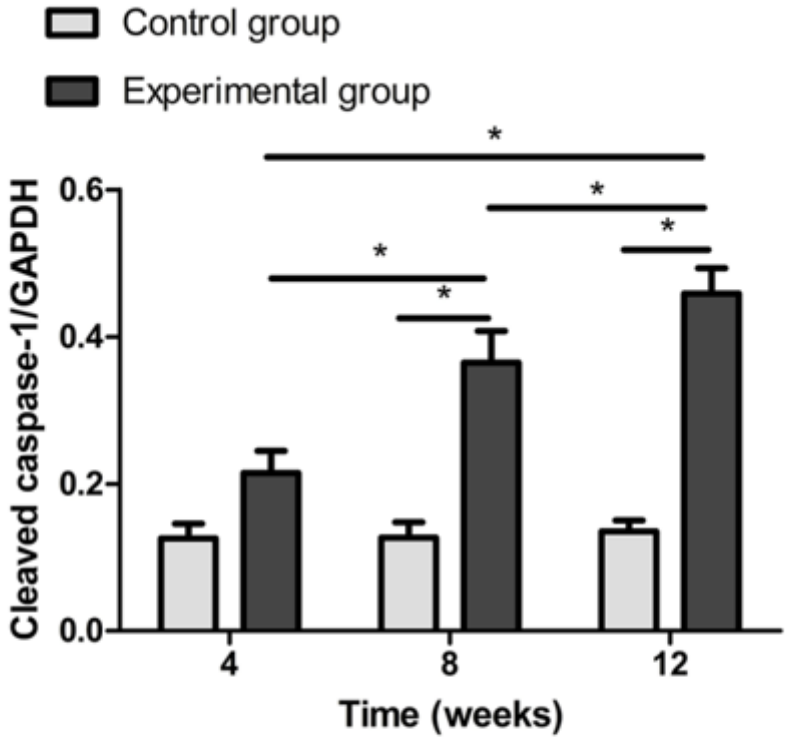


Figure 4

Analysis of the relative expression of caspase 1 between the control group and the experimental group using qRT-PCR. The values of the control group were considered to be 1. The relative expression of caspase 1 in the experimental group (4 w, 8 w, and 12 w) was obviously higher than that in the control group (**P < 0.01, the difference was statistically significant). However, no significant difference was established between the control group at 4 weeks, 8 weeks and 12 weeks (P >0.05).



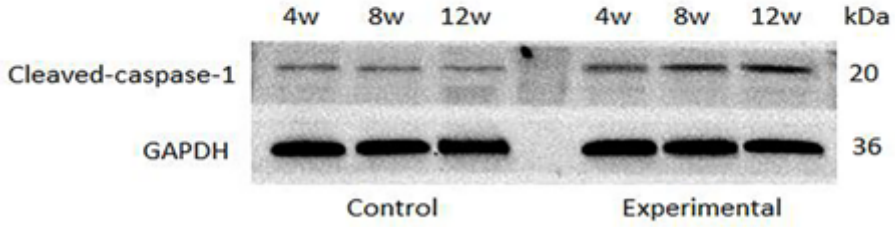


Figure 5

Analysis of the relative expression of Cleaved-caspase-1 in rat LECs between the experimental group and control group using Western blotting. Expression of each protein was normalized by GAPDH antibodies. There was no significant difference between the control groups at 4 weeks, 8 weeks and 12 weeks (P>0.05). There was no significant difference between the experimental group compared with the control group at 4 weeks (P=0.057). The relative expression of caspase-1 in the experimental group (8w and 12w groups) was obviously higher than that in control group. (*p < 0.05, the difference was statistically significant).

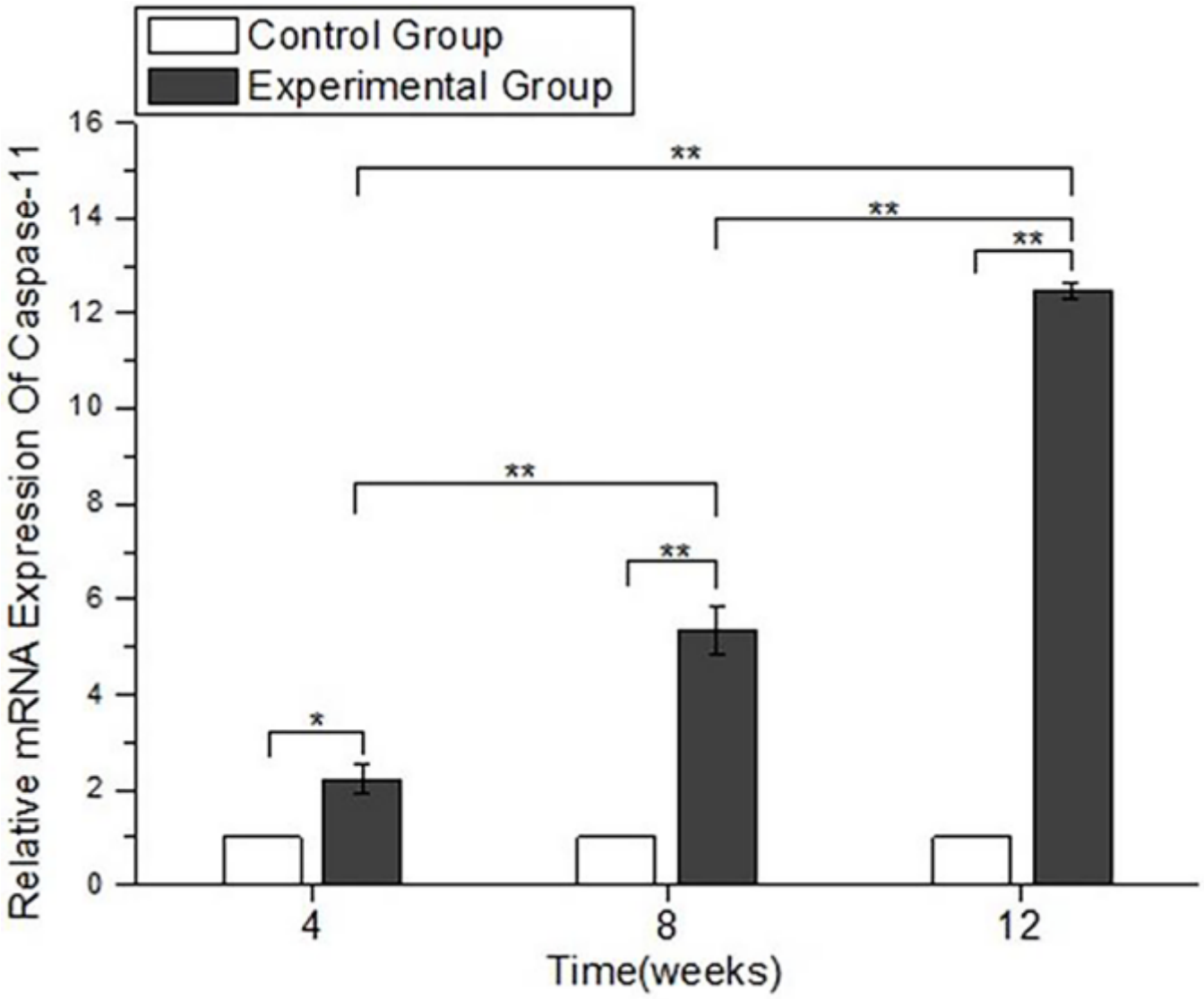


Figure 6

Analysis of the relative expression of caspase-11 between the control group and the experimental group using qRT-PCR. The values of control group were considered to be 1. The relative expression of caspase 11 in the experimental group (4w, 8w, and 12w) was obviously higher than that in the control group. (*$p < 0.05$, **$p < 0.01$, the difference was statistically significant).

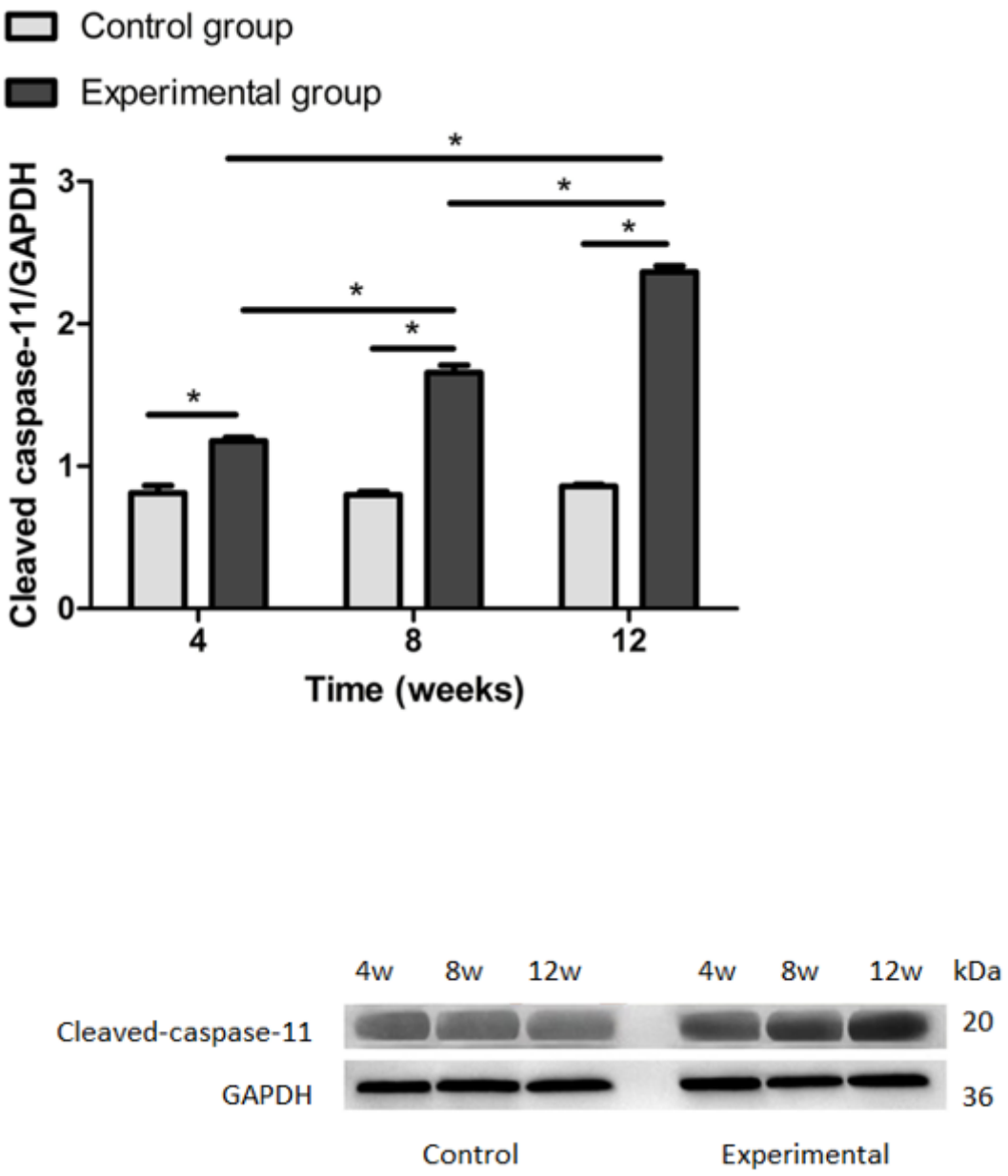


Figure 7

Analysis of the relative expression of Cleaved- caspase-11 between the experimental group and control group using Western blotting. Expression of each protein was normalized by GAPDH antibodies. The relative expression of Cleaved- caspase-11 in experimental group (4w, 8w, and 12w) was obviously higher than in control group. (*$p < 0.05$, the difference was statistically significant). However, no significant difference was established between the control group at 4 weeks, 8 weeks and 12 weeks ($p > 0.05$).

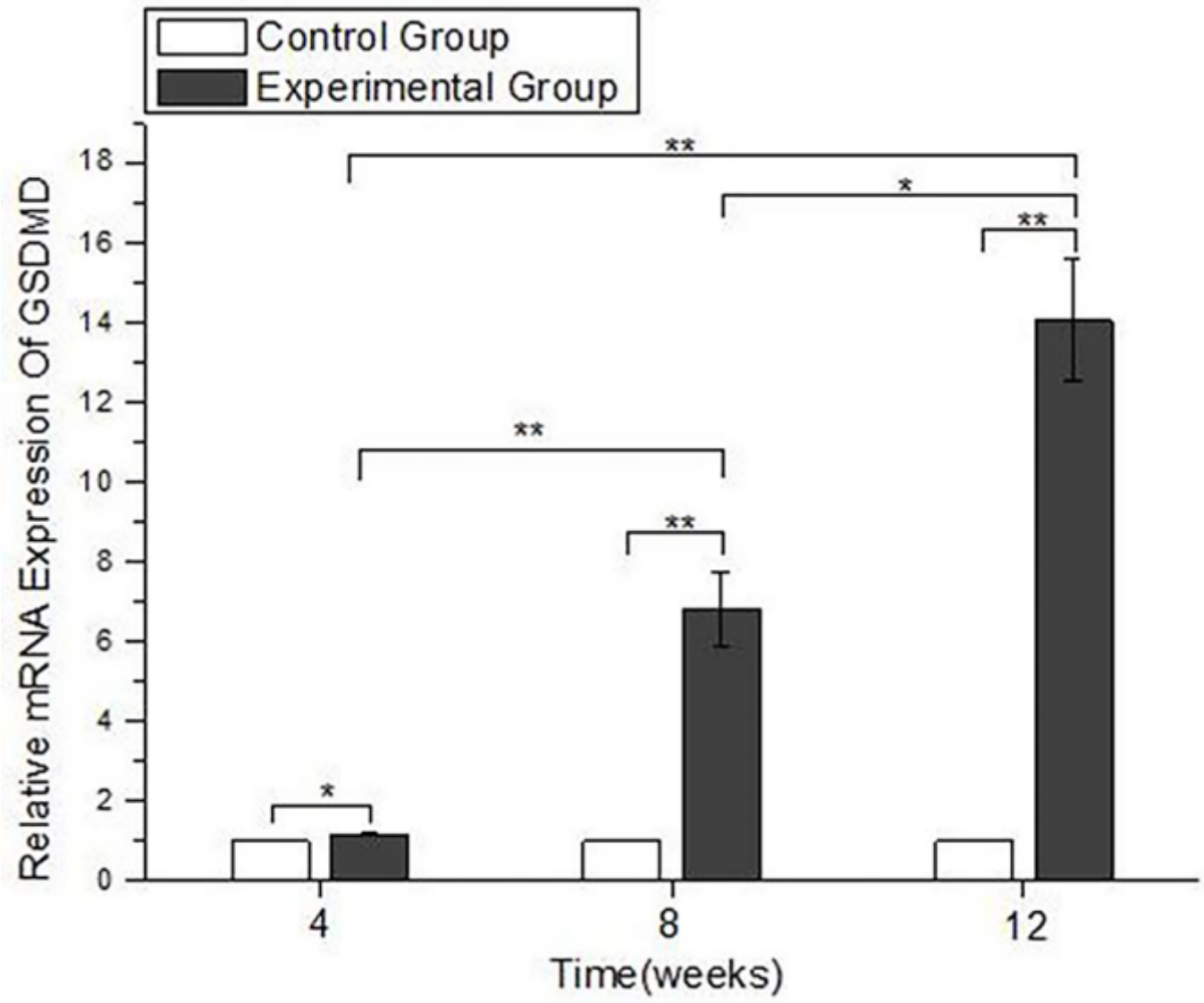


Figure 8

Analysis of the relative expression of GSDMD between the control group and the experimental group using qRT-PCR. The values of the control group were considered to be 1. The relative expression of GSDMD in the experimental group (4w, 8w, and 12w) was higher than that in the control group. (*$p < 0.05$, **$p < 0.01$, the difference was statistically significant), and the relative expression of GSDMD in the experimental group increased gradually with time.

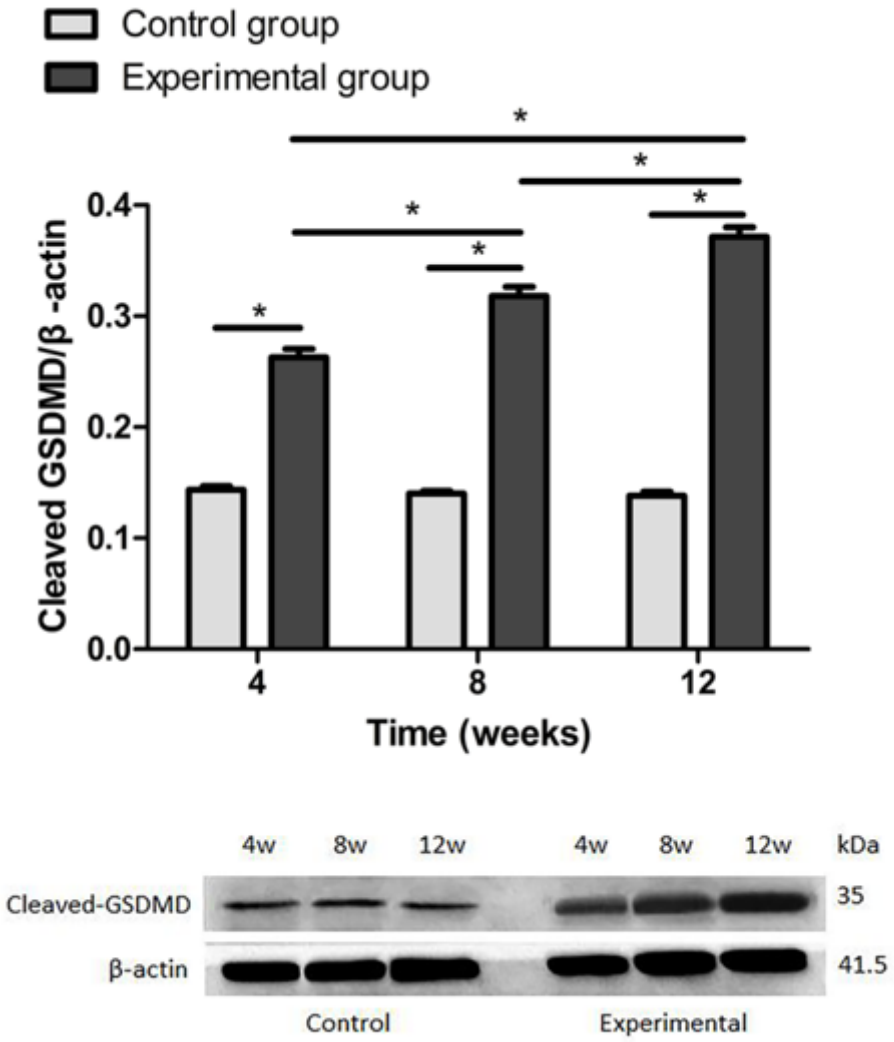


Figure 9

Analysis of the relative expression of GSDMD between the experimental group and control group using Western blotting. Expression of each protein was normalized by β-actin antibodies. The relative expression of cleaved-GSDMD in experimental group (4w, 8w, and 12w) was obviously higher than in control group. (*p < 0.05, the difference was statistically significant), and the relative expression of cleaved-GSDMD in the experimental group increased with time. However, no significant difference was established between the control group at 4 weeks, 8 weeks and 12 weeks (P >0.05).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- NC3RsARRIVEGuidelinesChecklist2014.docx
- SupplementaryMaterials.docx