Original Article

**Outer retinal involvement in N-methyl-D-aspartate-induced inner retinal injury in rabbits assessed by optical coherence tomography**

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**ABSTRACT** — This study was aimed to investigate morphological alteration of the retina with N-methyl-D-aspartate (NMDA)-induced injury in rabbits by optical coherence tomography (OCT). The right and left eyes of a total of 12 rabbits received single-intravitreal injection of vehicle and NMDA, respectively. Four out of the 12 animals underwent OCT and quantification of plasma microRNA repeatedly (4, 48, and 168 hr after dosing), followed by ocular histopathology at the end of the study. Ocular histopathology was also conducted in the eyes collected 4 or 48 hr after dosing from 4 animals at each time period. OCT revealed hyper-reflective ganglion cell complex and thickened inner retina in NMDA-treated eyes 4 hr after dosing; the inner retina shifted to thinning at later time points. The eyes given NMDA also exhibited greater thickness of the outer retina, which contains photoreceptors, after treatment, and thickened and obscured ellipsoid zone 168 hr after dosing. The plasma levels of miR-182 and miR-183, which are known to be highly expressed in photoreceptors, were higher 4 hr after dosing than pre-dosing values. Histopathologically, NMDA-induced inner retinal damage was confirmed: single-cell necrosis was observed in the ganglion cell layer and the inner nuclear layer 4 hr after dosing, the incidence of which decreased thereafter. At 168 hr after dosing, reduced number of ganglion cells was noted. No change was histopathologically observed in the outer retina. In conclusion, our results suggest involvement of photoreceptors in NMDA-induced inner retinal injury. Additionally, OCT revealed acute inner retinal findings suggestive of temporary edema.

**Key words:** N-methyl-D-aspartate, Intravitreal injection, Rabbits, Optical coherence tomography, Photoreceptors

**INTRODUCTION**

Excitotoxicity, which is induced by excessive glutamate stimulation to neurons through N-methyl-D-aspartate (NMDA) receptors, has been suggested to be the cause of disease in various vision-threatening inner retinal disorders such as retinal ischemia, proliferative diabetic retinopathy, and glaucoma, since it has been reported that glutamate level was elevated in the vitreous or aqueous fluid of eyes of patients with these disease conditions (Kageyama et al., 2000; Wakabayashi et al., 2006; Ambati et al., 1997; Simsek and Artunay, 2017; Dreyer et al., 1996; Honkanen et al., 2003). Major speculated mechanisms of the excessive glutamate are decreased uptake of glutamates in extracellular space by Muller glial cells through glutamate/aspartate transporters (GLASTs) and decreased conversion of glutamate to glutamine by glutamine synthetase in Muller glial cells (Ishikawa, 2013). Intravitreal injection of NMDA, a glutamate analog, causes cell death of inner retinal neurons including retinal ganglion cells (RGCs) in various animal species (Niwa et al., 2016). Therefore, the inner retinal insult induced by intravitreal NMDA in experimental animals is considered to be a good in vivo model to investigate pathophysiology of retinal diseases associated with excitotoxicity. Among them, the animal model of retinal injury induced by NMDA in rabbits, which have larger eyes than rodents (i.e., mice and rats), can be utilized to test
an experimental therapeutic intervention, and therefore has been characterized in terms of both histopathology (Hartveit et al., 1994; Kwong and Lam, 2000; Oku et al., 2004) and function (Oku et al., 2004).

Optical coherence tomography (OCT) is a noninvasive, high-resolution imaging technique to produce cross-sections of the ocular tissues including the retina and choroid, and therefore has advanced to become one of the most innovative and most successful translational optical imaging techniques in the last couple of decades, achieving substantial acceptance both in clinical ophthalmology (Drexler et al., 2014) and in animal research (Walther et al., 2011; McLellan and Rasmussen, 2012). However, there is limited evidence available for morphological characterization of the NMDA-induced retinal injury in rabbits by OCT. Therefore, this study was designed to investigate NMDA-induced excitotoxicity by using OCT in rabbits.

**MATERIALS AND METHODS**

**Animals**

A total of 12 male New Zealand White rabbits, purchased from Oriental Yeast Co., Ltd., were used in this study (15 weeks of age at the start of experiment). The animals were housed individually in aluminum/stainless steel cages (W 48.5 cm x D 77 cm x H 40 cm) in an animal study room where the environmental condition was set as follows: room temperature, 23°C; relative humidity, 55%; illumination, 12-hr lighting (7:00 to 19:00) at 150 to 300 luces. The animals were fed 100 g/animal/day of pellet food for rabbits (LRC4; Oriental Yeast Co., Ltd., Tokyo, Japan). Tap water from a feed-water nozzle was supplied *ad libitum* to the animals. All experimental procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Institutional Animal Care and Use Committee of Daiichi Sankyo Co. Ltd. (Tokyo, Japan; Approval number: 180513).

**Study design**

The animals were randomly assigned to one of three groups, as shown in Table 1. Animals in group 01 underwent a series of OCT and plasma microRNA (miRNA) measurements (4, 48 and 168 hr after intravitreal injection), followed by ocular histopathology 168 hr after injection. Animals in groups 02 and 03 were sacrificed 48 and 4 hr after injection, respectively, for ocular histopathology.

**Intravitreal injection of NMDA**

The animals were anesthetized with intramuscular injection of 10 mg/kg ketamine hydrochloride (Ketalar Intramuscular 500 mg; Daiichi Sankyo Co., Ltd.) and 0.1 mg/kg medetomidine hydrochloride (Domitor; Nippon Zenyaku Kogyo Co., Ltd. Fukushima, Japan). NMDA (Sigma-Aldrich, St. Louis, MO, USA) was dissolved at a concentration of 5.89 mg/mL (40 mM) in 0.1M phosphate buffered saline (PBS), followed by filter sterilization. After disinfection of the ocular surface using a mixture of polyvinyl alcohol iodine (PA-IODO ophthalmic and eye washing solution; Nitten Pharmaceutical Co., Ltd., Aichi, Japan), levofloxacin hydrate (Cravit ophthalmic solution 0.5%; Santen Pharmaceutical Co., Ltd., Osaka, Japan) and physiological saline, the eye was locally anesthetized with topical oxybuprocaine hydrochloride (Benoxil ophthalmic solution 0.4%; Santen Pharmaceutical Co., Ltd.), followed by generation of an ocular proptosis. The NMDA solution at a volume of 0.05 mL was injected into the vitreous of the left eye with a 30-gauge needle.
attached to a disposable syringe (Myshot; Nypro Corp., Osaka, Japan) inserted through the pars plana approximately 2-mm posterior to the dorso-nasal area of the limbus. An equal volume of PBS (0.05 mL) was injected into the right vitreous of each animal as vehicle control. After intravitreal injection, animals were treated with antibiotic eye drops (Cravit ophthalmic solution 0.5%; Santen Pharmaceutical Co., Ltd.) and with intramuscular injection of 0.5 mg/kg atipamezole hydrochloride (Antisedan; Nippon Zenyaku Kogyo Co., Ltd.) for quick recovery from general anesthesia.

**Clinical observation**

On the day of intravitreal injection, clinical signs were observed before and approximately 4 hr after injection. Thereafter, clinical signs were observed once a day in the morning until the day of necropsy.

**Spectral domain optical coherence tomography (SD-OCT)**

The animals were anesthetized with intramuscular injection of 10 mg/kg ketamine hydrochloride (Ketalar Intramuscular 500 mg; Daiichi Sankyo Co., Ltd.) and 0.1 mg/kg medetomidine hydrochloride (Domitor; Nippon Zenyaku Kogyo Co., Ltd.). The pupils were dilated with topical 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P ophthalmic solution; Santen Pharmaceutical Co., Ltd.); and the corneas were anesthetized with topical 0.4% oxybuprocaine hydrochloride (Benoxil ophthalmic solution 0.4%; Santen Pharmaceutical Co., Ltd.). To prevent the corneas from drying, rigid contact lenses (OOPS Inc., Kyoto, Japan) made of polymethyl methacrylate (Lens specifications: base curve, 8.0 mm; diameter, 12.8 mm; power, 0 D; central thickness, 0.2 mm) were placed on the corneal surface of both eyes. SD-OCT was performed with an RS-3000 Advance system (NIDEK Co. Ltd., Aichi, Japan), using a light source centered on 880 nm and with a rabbit-specific adapter installed. Each B-scan image consisted of 1024 A-scans. OCT images of the retina were collected from the outer edge of the inner nuclear layer thickness, 0.2 mm) were placed on the corneal surface of both eyes. SD-OCT was performed with an RS-3000 Advance system (NIDEK Co. Ltd., Aichi, Japan), using a light source centered on 880 nm and with a rabbit-specific adapter installed. Each B-scan image consisted of 1024 A-scans. OCT images of the retina were collected along the vertical meridians including the center of the optic nerve head. Each image was analyzed with respect to outer retinal thickness, inner nuclear layer thickness, and ganglion cell complex (GCC) thickness using a Java-based image processing program (ImageJ 1.50i; National Institutes of Health, MD, USA). Outer retinal thickness was measured from the tips of the outer segments to the inner edge of the outer nuclear layer (ONL), and GCC thickness was measured from the outer edge of the inner plexiform layer (IPL) to the vitreal surface.

**Quantification of plasma microRNA (miRNA) level**

The absolute values of circulating miRNAs were quantified as described previously (Iguchi et al., 2018). Approximately 0.5 mL blood samples were collected from the auricular vein immediately before the SD-OCT and placed in tubes containing EDTA-2K (Microtainer; Becton, Dickinson and Co., Franklin Lakes, NJ, USA). The plasma was prepared from the blood samples by centrifugation at 10,000 g for 10 min at 4°C. Total RNA was extracted from 200 μL of the plasma samples using miRNeasy mini kit (Qiagen, Venlo, Netherlands) according to the manufacturer’s protocol. The total RNA samples were reverse-transcribed using a TaqMan MicroRNA Reverse Transcription kit (Thermo Fisher Scientific) with a target miRNA RT primer of the TaqMan MicroRNA Assays (Thermo Fisher Scientific). The samples were preamplified using a Taqman PreAmp Master Mix (Thermo Fisher Scientific) with a GeneAmp PCR System 9700 according to the manufacturer’s instructions. Then, the target miRNAs, miR-182 and miR-183 (Assay IDs: 002334 and 002269, respectively), were applied to qPCR with TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific) on a 7900HT Fast Real Time PCR System (Thermo Fisher Scientific). The thermal cycling conditions were 50°C for 2 min, then 95°C for 20 sec, followed by 45 cycles of 95°C for 1 sec and 60°C for 20 sec. The expression levels of miR-182 and miR-183 were normalized to cel-miR-238 (Syn-cel-miR-238 miScript miRNA Mimic, Qiagen) that had been spiked-in during the extraction as an external control.

**Pathology**

Animals were euthanized by exsanguination from the abdominal aorta and postcava after intravenous anesthesia with 35 mg/kg pentobarbital sodium (Somnopenyl; Kyoritsu Seiyaku Co., Tokyo, Japan). Thereafter animals were necropsied and examined macroscopically. The eyes were collected and fixed with Davidson’s fluid. The fixed eyes were embedded in paraffin, sectioned vertically through the optic nerve (superior-inferior), and stained with hematoxylin and eosin (H&E). In addition, immunohistochemistry was performed by the immunoenzyme polymer method (EnVision kits; Dako, Agilent Technologies, Inc., Santa Clara, CA, USA). Mouse monoclonal antibodies against calretinin (clone 6B8.2; Merck Millipore, Burlington, MA, USA), calbindin (clone D-28K; Sigma-Aldrich CO. LLC., St. Louis, MO, USA), PKC alpha (clone MC5; Novus Biologicals, Centennial, CO, USA) or vimentin (clone V9; Dako) were used as the primary antibody. The sections were stained with diaminobenzidine and counterstained with Mayer’s hematoxy-
lin. All slides were examined under a light microscope.

**Statistics**

For statistical analysis of the OCT parameters (i.e., thickness of retinal layers), the mean and standard deviation (S.D.) were calculated for the vehicle- and NMDA-treated eyes at each time point. Repeated measures two-way ANOVA was used to assess the difference between the vehicle- and NMDA-treated eyes, followed by Aspin-Welch’s t-test only if statistical significance was observed not only on the group comparison but also on the interaction between group and time course. For statistical analysis of the plasma miRNA level, measured values were converted to decadic logarithm, and the mean and S.D. were calculated at each time point. Paired t-test with Bonferroni’s correction was used to assess the difference between the pre- and post-dosing values. The differences were considered to be significant when \( P \) was less than 0.05.

**RESULTS**

**Clinical observation**

All NMDA-treated eyes showed myosis 4 hr after injection, which persisted for up to 2 days (Table 1). Thereafter, no abnormal clinical signs including any suggestive of visual or neural disturbance were observed in any animal.

**SD-OCT**

Compared to PBS-treated eyes, NMDA-treated eyes exhibited hyper-reflective GCC, thickened IPL, and greater GCC thickness 4 hr after dosing (Fig. 1B, F). At later time points, the IPL shifted to thinning (Fig. 1D, H) and the GCC thickness in NMDA-treated eyes was less than that in PBS-treated eyes (\( P < 0.01 \), Fig. 2A). Moreover, the NMDA-treated eyes showed greater outer retinal thickness after dosing (\( P < 0.01 \), Fig. 2C) and thickened and obscured ellipsoid zone 168 hr after dosing (Fig. 1D, H), indicating abnormality in the outer retina.

**Plasma miRNA level**

To demonstrate the effect of NMDA on photoreceptors, which was detected by OCT, from a different angle, we quantified plasma miR-182 and miR-183, which are miRNAs reported to be associated with photoreceptors (Xu et al., 2007). Compared to pre-dosing values, the plasma levels at 4 hr after dosing with NMDA were significantly higher both for miR-182 (\( P < 0.05 \)) and miR-183 (\( P < 0.01 \)) (Fig. 3), suggesting photoreceptor involvement.

**Pathology**

Pathological findings are shown in Table 2. Macroscopically, mild hydrocephalus was noted in 1 animal (No. 01M03). This finding was judged as spontaneous and not to affect the conclusion of the present study.

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**Fig. 1.** Representative OCT images after intravitreal injection of NMDA in rabbits. PBS and NMDA at a dose of 2 μmol/eye were intravitreally injected to the right (A to D) and left (E to H) eyes, respectively. OCT images of sagittal section through the retina including the optic nerve head were obtained at pre-dosing (A, E), and 4 hr (B, F), 48 hr (C, G) and 168 hr (D, H) after dosing. Hyper-reflectivity of GCC and thickening of IPL were observed 4 hr after dosing of NMDA (asterisk). At 168 hr after NMDA dosing, thinning of IPL (arrow head) and thickening and obscuration of EZ (arrow) were observed. Abbreviations: GCC, ganglion cell complex; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; EZ, ellipsoid zone; RPE, retinal pigment epithelium.

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since there was no other abnormal finding in any examination for this animal. Microscopically, NMDA-induced inner retinal damage was confirmed: mild single-cell necrosis was observed in the ganglion cell layer (GCL) and the innermost portion of inner nuclear layer (INL) 4 hr after dosing (Fig. 4A, D), the incidence of which decreased thereafter. The necrotic cells in the INL were mainly co-localized with calretinin-positive cells, indicating predominant involvement of Amacrine cells in the INL (Fig. 5A). Mononuclear cell infiltration in the vitreous body suggestive of a reaction to the retinal damage was noted 48 and 168 hr after dosing. At 168 hr after dosing, reduced number of ganglion cells was observed (Fig. 4C, F).

**DISCUSSION**

This study was aimed to investigate morphological changes of the retina in a rabbit NMDA-induced inner retinal injury model by means of SD-OCT, a clinically relevant *in vivo* retinal imaging technology. Unexpectedly, we observed interesting OCT findings of the outer retina as well as those of the inner retina. In rabbit eyes injected with NMDA, thickness of the outer retina, which is mainly composed of photoreceptors, was slightly greater than in control eyes after treatment. Additionally, the...
ellipsoid zone, which has been reported to correspond to a mitochondria-rich region of photoreceptor inner segments (Xie et al., 2018), became thickened and obscured in NMDA-injected eyes. The ellipsoid zone abnormality was preceded by the findings of the inner retina, and no direct evidence of NMDA receptor expression on photoreceptors in rabbits has been reported. It is, therefore, speculated that photoreceptors were affected secondarily to the NMDA-induced inner retinal cell death. Although further study is definitely needed to elucidate how NMDA exerts an effect on photoreceptors in rabbits, the OCT findings in this study indicate involvement of photoreceptors in the rabbit NMDA-induced inner retinal injury model. Meanwhile, we observed no apparent abnormality of the outer retina in histopathology, which was consistent with previous researches using rabbits (Kwong and Lam, 2000; Oku et al., 2004). The exact reason for the absence of outer retinal abnormality in histopathology is unclear; however, we speculated that ionic balance between intra- and extra-cellular spaces in the outer reti-

Fig. 3. Time course of changes in plasma miRNA levels after intravitreal injection of NMDA in rabbits. PBS and NMDA at a dose of 2 μmol/eye were intravitreally injected to the right and left eyes, respectively, and serial plasma concentrations of miR-182 and miR-183 were quantified. Data are expressed as mean +/- S.D. of four animals. Significantly higher values in comparison with the pre-dosing values were observed both in miR-182 (*P < 0.05) and miR-183 (**P < 0.01) by the paired t-test with Bonferroni’s correction.

Fig. 4. Representative photomicrographs of the retina from PBS- and NMDA-treated eyes in rabbits. PBS and NMDA at a dose of 2 μmol/eye were intravitreally injected to the right and left eyes, respectively. The PBS-treated eyes (A to C) and NMDA-treated eyes (D to F) were collected at 4 hr (A, D), 48 hr (B, E) and 168 hr (C, F) after dosing. Note single-cell necrosis in GCL and INL at 4 and 48 hr after dosing (arrow heads) and decreased number of ganglion cells at 168 hr after dosing (arrows) in NMDA-treated eyes. Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Hematoxylin and eosin stain.
was disturbed by NMDA, which could not be detected
histopathologically. Another possible explanation is that a
slight histopathological finding was masked by artifacts
created during preparation of histopathological speci-
mens. To approach the effect of NMDA on photoreceptors
from a different angle, we quantified miR-182 and miR-
183, which are reported to be highly expressed in pho-
toreceptors (Xu et al., 2007) and to play a pivotal role in
photoreceptor maturation and maintenance (Xiang et al.,
2017), in plasma samples collected just prior to each OCT
examination in this study. As a result, we observed mild-
ly increased level of both plasma miR-182 and miR-183.
We have confirmed that an intravitreal injection of vehicle
alone induces no significant change in miR-182 or miR-
183 in rabbits (data is not shown). Therefore, this find-
ing in plasma miRNAs is also suggestive of outer retinal
involvement, which is in alignment with the OCT find-
ings in the outer retina mentioned above. To our knowl-
edge, this is the first report to describe the involvement of
photoreceptors in inner retinal injury induced by intravit-
real injection of NMDA. However, further study is defi-
nitely needed to elucidate how NMDA exerts an effect on
photoreceptors. Although it has been reported that intrav-
itreal injection of NMDA to rabbits did not affect a-wave
of dark-adapted electroretinogram (ERG), which mainly
originates from rod photoreceptors (Oku et al., 2004),
more detailed ERG assessment focused on a specific out-
er retinal function (e.g., phototransduction, photoreceptor
deactivation, and dark adaptation) might reveal functional
impairment of photoreceptors.

As acute changes in the retina, we observed transient
thickening of the IPL 4 hr after intravitreal injection of
NMDA by OCT. It has been reported that expression of
NR2A protein, a subunit of the NMDA receptor, in the
retina was localized to the IPL in several animal species
including rabbits (Hartveit et al., 1994). This specific dis-
tribution of the NMDA receptors in the retina is consist-
ent with the acute thickening reversibly induced by their
ligand (i.e., NMDA) and observed by OCT in this study.
Therefore, the aforementioned OCT finding in rabbits in
the present study is suggestive of transient edema in the
inner retina resulting from accumulation of NMDA mole-
ules within the IPL and/or disturbed ionic balance via an
excess excitement of NMDA receptors, which are iono-
tropic. In addition, all rabbits in this study exhibited uni-
lateral myosis of NMDA-injected eye 4 hr after dosing,
the incidence of which decreased at later time points.
This reversible clinical finding might also be attributable
to the transient hyper-excitability of the third-order retinal
neurons (i.e., RGCs), which results in excessive output
of visual information to the optic nerve, therefore induc-
es persistent excitement of the ipsilateral Edinger-West-
phal nucleus and its efferent pathway of light reflex. We
observed OCT changes in the inner retina at later time

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Fig. 5. Photomicrographs of immunohistochemistry for retinal components, nuclei of which are housed in the INL, in NMDA-
injected rabbit eye. The retina collected 4 hr after NMDA dosing was labeled with calretinin (A, Amacrine cells), calbindin
(B, horizontal cells), PKC alpha (C, bipolar cells) or vimentin (D, Muller glial cells). The necrotic cells in the INL was
mainly co-localized with calretinin-positive cells (arrowheads), indicating predominant involvement of Amacrine cells in
the INL. Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; RPE, retinal pigment
epithelium. Immunostaining.
points as well: qualitatively thinned IPL and quantitatively decreased thickness of the GCC were observed in NMDA-treated eyes by SD-OCT 48 hr after dosing or later. These findings reflected histopathological lesions that illustrate decreased neural component of the inner retina such as decreased number of nuclei in the GCL, which was in agreement with previous reports where rabbit eyes were injected with NMDA and examined histopathologically (Kwong and Lam, 2000; Oku et al., 2004).

In histopathological examination, we observed single-cell necrosis in both the GCL and the innermost part of the INL, which were consistent with a previous report in rabbits (Kwong and Lam, 2000). This localization of NMDA-induced damaged cells in rabbit retinas matches well with that of mRNA expression of NR2A, a subunit of the NMDA receptor, reported in rat retina (Hartveit et al., 1994). Therefore, these results support the proposal that NMDA-induced retinal injury is an excitotoxicity, where cell death is caused by the uptake of excessive glutamate via NMDA receptors (Lipton and Rosenberg, 1994). To make a more in-depth investigation into the affected cell types in the INL, we performed immunohistochemistry of NMDA-treated retinas for four types of retinal cells, of which nuclei are located in the INL: calretinin for Amacrine cells (Huang et al., 2013), calbindin for horizontal cells (Huang et al., 2013), PKC alpha for retinal bipolar cells (Huang et al., 2013), and vimentin for Muller glial cells (Bringmann et al., 2006). As a result, necrotic cells in the innermost part of the INL were calretinin positive. It has been reported that functional NMDA receptors are expressed by some subtypes of Amacrine cells in the mammalian retina (Zhou et al., 2016). These findings indicate Amacrine cell death in the INL is attributable to the excitotoxicity via NMDA receptors as well as RGCs in the GCL in the NMDA-treated rabbit retinas in this study.

In conclusion, our results suggest involvement of photoreceptors in the rabbit NMDA-induced inner retinal injury model, which exhibits marked cell death of RGCs and Amacrine cells. Additionally, OCT revealed acute inner retinal findings suggestive of temporary edema.

Table 2. Pathological findings.

| Test article | Dose (μmol/eye) | Group | Time after injection | Findings | Gross pathology | Histopathology |
|--------------|----------------|-------|---------------------|----------|----------------|----------------|
|              | 0              | 03    | 4 hr (Day 1)        | +        | Hydrocephalus   | Mononuclear cell infiltration in the vitreous body |
|              | 0              | 02    | 48 hr (Day 3)       | +        |                | Single cell necrosis in the retinal ganglion cell layer |
|              | 0              | 01    | 168 hr (Day 8)      | +        |                | Decreased number of retinal ganglion cells |
|              | 0              | 03    | 4 hr (Day 1)        | +        | Hydrocephalus   | Mononuclear cell infiltration in the vitreous body |
|              | 2              | 02    | 48 hr (Day 3)       | +        |                | Single cell necrosis in the retinal ganglion cell layer |
|              | 2              | 01    | 168 hr (Day 8)      | +        |                | Decreased number of retinal ganglion cells |
|              |                |       |                     |          |                | Single cell necrosis in the inner nuclear layer |

Numerals represent the number of animals (Gross pathology) or eyes (Histopathology)
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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Ambati, J., Chalam, K.V., Chawla, D.K., D’Angio, C.T., Guillette, E.G., Rose, S.J., Vanderlinde, R.E. and Ambati, B.K. (1997): Elevated gamma-aminobutyric acid, glutamate, and vascular endothelial growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. Arch. Ophthalmol., 115, 1161-1166.

Bringmann, A., Pannicke, T., Grosche, J., Francke, M., Wiedemann, P., Skatchkov, S.N., Osborne, N.N. and Reichenbach, A. (2006): Müller cells in the healthy and diseased retina. Prog. Retin. Eye Res., 25, 397-424.

Drexler, W., Liu, M., Kumar, A., Kamali, T., Unterhuber, A. and Leitgeb, R.A. (2014): Optical coherence tomography today: speed, contrast, and multimodality. J. Biomed. Opt., 19, 071412.

Dreyer, E.B., Zurakowski, D., Schmer, R.A., Podos, S.M. and Lipton, S.A. (1996): Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. Arch. Ophthalmol., 114, 299-305.

Hartvet, E., Brandstätter, J.H., Sassolé-Poggetto, M., Laurie, D.J., Seeburg, P.H. and Wäsche, H. (1994): Localization and developmental expression of the NMDA receptor subunit NR2A in the mammalian retina. J. Comp. Neurol., 348, 570-582.

Honkanen, R.A., Baruah, S., Zimmerman, M.B., Khanna, C.L., Weaver, Y.K., Narkiewicz, J., Waziri, R., Gehrs, K.M., Weingeist, T.A., Boldt, H.C., Folk, J.C., Russell, S.R. and Kwon, Y.H. (2003): Vitreous amino acid concentrations in patients with glaucoma undergoing vitrectomy. Arch. Ophthalmol., 121, 183-188.

Huang, J., Zhou, L., Wang, H., Luo, J., Zeng, L., Xiong, K. and Chen, D. (2013): Distribution of thrombospondins and their neuronal receptor q251 in the rat retina. Exp. Eye Res., 111, 36-49.

Iguchi, T., Nino, N., Tamai, S., Sakurai, K. and Morii, K. (2018): Absolute Quantification of Plasma MicroRNA Levels in Cynomolgus Monkeys, Using Quantitative Real-time Reverse Transcription PCR. J. Vis. Exp., 132, e56850.

Ishikawa, M. (2013): Abnormalities in glutamate metabolism and excitotoxicity in the retinal diseases. Scientifica (Cairo), 2013, 528940.

Kageyama, T., Ishikawa, A. and Tamai, M. (2000): Glutamate elevation in rabbit vitreous during transient ischemia-reperfusion. Jpn. J. Ophthalmol., 44, 110-114.

Kwong, J.M. and Lam, T.T. (2000): N-methyl-D-aspartate (NMDA) induced apoptosis in adult rabbit retinas. Exp. Eye Res., 71, 437-444.

Lipon, S.A. and Rosenberg, P.A. (1994): Excitatory amino acids as a final common pathway for neurologic disorders. N. Engl. J. Med., 330, 613-622.

McLellan, G.J. and Rasmussen, C.A. (2012): Optical coherence tomography for the evaluation of retinal and optic nerve morphology in animal subjects: practical considerations. Vet. Ophthalmol., 15 (Suppl 2), 13-28.

Niwa, M., Aoki, H., Hirata, A., Tomita, H., Green, P.G. and Hara, A. (2016): Retinal Cell Degeneration in Animal Models. Int. J. Mol. Sci., 17, 110.

Oku, H., Goto, W., Okuno, T., Kobayashi, T., Sugiyama, T., Ota, T., Yoneda, S., Hara, H. and Ikeda, T. (2004): Effects of poly(ADP-ribose) polymerase inhibitor on NMDA-induced retinal injury. Curr. Eye Res., 29, 403-411.

Simsick, I.B. and Artunay, O. (2017): Evaluation of Biochemical Composition of Vitreous of Eyes of Diabetic Patients Using Proton Magnetic Resonance Spectroscopy. Curr. Eye Res., 42, 754-758.

Wakabayashi, Y., Yagihashi, T., Kezuka, J., Muramatsu, D., Usui, M. and Iwasaki, T. (2006): Glutamate levels in aqueous humor of patients with retinal artery occlusion. Retina, 26, 432-436.

Walther, J., Gaertner, M., Cimalla, P., Burkhardt, A., Kirsten, L., Meissner, S. and Koch, E. (2011): Optical coherence tomography in biomedical research. Anal. Bioanal. Chem., 400, 2721-2743.

Xiang, L., Chen, X.J., Wu, K.C., Zhang, C.J., Zhou, G.H., Lv, J.N., Sun, L.F., Cheng, F.F., Cai, X.B. and Jin, Z.B. (2017): miR-183/96 plays a pivotal regulatory role in mouse photoreceptor maturation and maintenance. Proc. Natl. Acad. Sci. USA, 114, 6376-6381.

Xie, W., Zhao, M., Tsai, S.H., Burkes, W.L., Potts, L.B., Xu, W., Payne, H.R., Hein, T.W., Kuo, L. and Rosa, R.H. Jr. (2018): Correlation of spectral domain optical coherence tomography with histology and electron microscopy in the porcine retina. Exp. Eye Res., 177, 181-190.

Xu, S., Witmer, P.D., Lumayag, S., Kovacs, B. and Valle, D. (2007): MicroRNA (miRNA) transcriptome of mouse retina and identification of a sensory organ-specific miRNA cluster. J. Biol. Chem., 282, 25053-25066.

Zhou, Y., Tencerová, B., Hartvet, E. and Veruki, M.L. (2016): Functional NMDA receptors are expressed by both AII and A17 amacrine cells in the rod pathway of the mammalian retina. J. Neurophysiol., 115, 389-403.