

Establishment of a Rapid, Lesion-Controllable Retinal Degeneration Model in Monkey for Preclinical Stem Cell Therapy

Guanjie Gao

Sun Yat-Sen University Zhongshan Ophthalmic Center

Liwen He

Sun Yat-Sen University Zhongshan Ophthalmic Center

Shengxu Liu

Sun Yat-Sen University Zhongshan Ophthalmic Center

Dandan Zheng

Sun Yat-Sen University Zhongshan Ophthalmic Center

Xiaojing Song

Sun Yat-Sen University Zhongshan Ophthalmic Center

Wenxin Zhang

Sun Yat-Sen University Zhongshan Ophthalmic Center

Minzhong Yu

University Hospitals

Guangwei Luo

Sun Yat-Sen University Zhongshan Ophthalmic Center

Xiufeng Zhong (✉ Zhongxf7@mail.sysu.edu.cn)

Sun Yat-Sen University

Research

Keywords: animal model, monkey, retinal degeneration, sodium nitroprusside, stem cell therapy

Posted Date: August 17th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-59826/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Cells on November 13th, 2020. See the published version at <https://doi.org/10.3390/cells9112468>.

Abstract

Background: Retinal degenerative disorders (RDs) are the main cause of blindness without curable treatment. Our previous studies have demonstrated that human induced pluripotent stem cells can differentiate into retinal organoids with all subtypes of retina, which provides huge promises for treating these diseases. Before it can be turned into reality, RD animal models are required to evaluate the safety and efficacy of stem cell therapy, and to develop the surgical tools and procedures for cell transplantation in patients. This study is to develop a monkey model of RD with controllable of lesion sites which can be rapidly prepared, for the studies of preclinical stem cell therapy among other applications.

Methods: Sodium nitroprusside (SNP) in three doses was delivered into the monkey eye by subretinal injection (SI) and normal saline was applied as control. Structural and functional changes of the retinas were evaluated via multimodal imaging techniques and multifocal electroretinography (mfERG) before and after the treatment. Histological examination was performed to identify the target layer of the affected retina. The health status of monkeys was monitored during the experiment.

Results: Well defined lesion with various degree of retinal degeneration was established at the posterior pole of retina as early as 7 days after SNP SI. The damage effect of SNP was dose-dependent. 0.05 mM SNP caused invisible structural changes in retina, similar to the control. 0.1 mM SNP led to the loss of outer retinal layer, including OPL, ONL and RPE, while 0.2 mM SNP impacted the entire layer of retina and choroid. MfERG showed reduced amplitude in the damaged region. The structural and functional damages were not recovered after 7-month follow-up.

Conclusion: A simple, rapidly induced, lesion site-controllable, retinal degeneration model in monkey was established by the subretinal injection of 0.1 mM SNP. This monkey model closely mimics the histological changes of RDs, and provides a valuable platform for preclinical assessment of stem cell therapy.

Background

Retinal degenerations (RD) with different pathogeneses, such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP), may cause the dysfunction or degeneration of retinal pigment epithelium (RPE) and/or photoreceptor, and finally cause blindness[1, 2]. Despite various therapeutic modalities developed to slow cell death progress or restore their functions [3–5], there is no cure for these diseases so far. Retinal cell transplantation has been regarded as a potential treatment to replace the damaged cells, and to restore their structure and function as well[6]. With the rapid development of human pluripotent stem cell (hPSC) technology and successful reproduction of retinal cells and tissues with hPSCs [7–12], the bottleneck of retinal grafts, cell or tissue donor issue, has been largely solved. Therefore, stem cell therapy holds huge promises for restoring the vision of RD patients.

RD animal models are required to evaluate the safety and efficacy of stem cell therapy, and to develop the surgical tools and procedures for cell transplantation in patients[13]. To this end, animals with eyeball

size and structure closely similar to human counterparts would be preferred. Nonhuman primates like monkeys are one of such animals since they have not only the equivalent eyeball diameter, but also the macula, a unique structure of primates' retina, responsible for detailed, daytime vision and color vision[14]. Due to the high cost in purchasing and breeding these animals, the establishment of a RD model in monkeys which can be rapidly prepared, and is highly reproducible and closely relevant to the clinical settings with low lethality, is largely in demand.

Retinal degenerative conditions in large-sized animals such as cats, rabbits, pigs and monkeys have been induced by several approaches including laser or severe light exposure, and retinotoxic reagents[15–22]. However, some limitations exist in these models. For instance, laser or light exposure caused focal damage of retina with small lesion size or inconsistent damage degree[18, 19]; intravenous injection of retinotoxic agents such as sodium iodate and N-methyl-N-nitrosourea (MNU) led to systemic complications with high mortality, while intravitreal injection of the above agents caused uncontrollable or random distribution of retinal lesions[13, 20–22]. A new method of subretinal injection (SI) has been applied to deliver cobalt chloride to induce a lesion-controllable RP model in monkeys for retinal sheet transplantation[17]. But the characteristics of this type of focal RD models is unclear.

Recently, several groups including us have reported that sodium nitroprusside (SNP) is a safe agent in generating rabbit model[23, 24]. In addition, the SNP has been used to treat patients with hypertension. Therefore, here we subretinally delivered SNP into the posterior pole of retina in monkeys. The dose-effect of SNP on retinal damage was evaluated in structural and functional level by multi-modalities. A focal, perimacular RD model in monkeys has been established, which is suitable for the test of new therapeutics, stem cell therapy in particular.

Materials And Methods

Animals

The study design and experimental protocols were approved by the Application Format of Animal Experimental Ethical Inspection to Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-Sen University. All experimental procedures involving animals adhered to the Association Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. 14 healthy male cynomolgus monkeys (28 eyes) aged 1-4 years were used in this study, and housed individually in stainless steel cages in an animal experimental room with the environmental conditions at 16-26°C room temperature, 40-70 % humidity and 12 h lighting (7 AM to 7 PM; illumination intensity >200 lux). The animals were generally fed 5 % body weight/animal/day of pellet food which was adapted for the changes in appetite and weight of monkeys. Tap water from a feed-water nozzle was provided ad libitum to the animals.

Drug delivery

The SNP (Hongyuan, China) was dissolved in normal saline (NS) at three different concentrations (0.05 mM, 0.1 mM, 0.2 mM) followed by filter sterilization, and protected from light. The cynomolgus monkeys were anesthetized with intramuscular injection of 4-6 mg/kg (50 mg/mL) Zoletil 50 (Virbac, France) and topical application of 0.5 % proparacaine hydrochloride eyedrops (Ruinian Best, China). The pupils were fully dilated (≥ 7 mm) using 0.5 % tropicamide phenylephrine eye drops (Mydrin, Santen) before operation. Under a surgical microscope (M844F20, Leica, Germany), 100 μ L SNP solution of different concentrations or NS was injected into the subretinal space at the posterior pole of retina next to the fovea via a 34 G needle (NF34BL-2, WPI, China), respectively. Health indicators, such as weight and diet, were monitored during the experimental period.

***In vivo* observation and evaluation**

The morphological changes of the retina were observed with a binocular indirect ophthalmoscope (HEINE OMEGA 500, HEINE Optotechnik GmbH & Co. KG, Germany) and photographed with a digital fundus camera (TRC-50DX, Japan Topcon, Japan).

Spectral-domain optical coherence tomography (SD-OCT) images were acquired with the Spectralis HRA+OCT (Heidelberg Engineering, Germany) in high-speed mode with a 30° \times 25° horizontal line scan with 9 frames averaged in each B scan. The total retinal thickness in the injection site within a circle of 6 mm in diameter was measured.

BluePeak-autofluorescence (BAF) images were acquired using a confocal scanning laser ophthalmoscope (cSLO) which had a 488 nm excitation filter and a 500 nm barrier filter, and equipped with an internal fluorescent reference for the correction of variable laser power and differences in detector sensitivity.

Fluorescein angiography (FA) and indocyanine green angiography (ICGA) were performed with cSLO before the operation and 5-month after SNP injection. The cSLO had a 785 nm excitation filter and an 820 nm barrier filter for ICGA, and had a 488 nm excitation filter and a 500 nm barrier filter for FA. We administered 0.5 ml/kg of 20% sodium fluorescein (Baiyunshan, China) or 2.5 mg/kg of indocyanine green (ICG; Ruidu, China) intravenously at each attempt. FA was performed first and was completed in 15 min. Next, ICGA was performed and was recorded in approximately 40 min for each eye. Videos of the first minute of FA and ICGA were recorded, and pictures of the angiograms were taken every 15 s after 1min from the beginning.

Histological evaluation

The monkeys were euthanized using a lethal dose of potassium chloride (35 mg/kg) injected into the elbow vein 7-month after SNP injection. Right after death, both eyes were enucleated and immersed in a mixture of 10 % neutral-buffered formalin, and embedded in paraffin. 5 μ m-thick sections of the injected area were cut and stained with hematoxylin and eosin (H&E) and recoverin (1:500, millipore, USA). The

slides were examined to detect pathological changes in the retina using a light microscope (Axio Scan Z1, Zeiss, Germany).

Functional evaluation

The functional changes of SNP treated retinal lesions were evaluated with noninvasive multifocal electroretinogram (mfERG) with RETImap system (Roland Consult, Brandenburg, Germany) following the International Society of Visual Clinical Electrophysiology (ISCEV) standards[25]. The pupils were fully dilated (≥ 7 mm) using 0.5 % tropicamide phenylephrine (Mydrin, Santen). A gold foil annular corneal active electrode was put onto an anesthetized cornea in each test; a ground electrode was placed to the skin on the forehead; and a reference electrode was placed near the orbital rim. In a typical recording, the fundus was visualized using SLO, and the stimulus pattern consistently positioned within the injection area of retina. After each stimulus cycle, a fundus photograph from the SLO was taken to record the corresponding fundus position. An array of 37 unscaled hexagons with the total diameter of about 25 degrees of arc was projected onto the retina in the central area of macula under infrared fundus monitoring. The mfERG tests were repeated 6 cycles. Right eye and left eye were tested one by one. After completion of the test, tobramycin eye drops were placed in each eye.

Statistical analysis

Results are presented as mean \pm standard deviation (SD). Data were analyzed using t-test. Statistical analysis was performed using GraphPad Prism software (GraphPad Software, San Diego, CA). The criterion for statistical significance was $P < 0.05$.

Results

Subretinal administration of SNP induced controllable, focal retinal degeneration in cynomolgus monkeys

To overcome the lethal side effect of systemic delivery of retinotoxic reagents or random or uneven distribution of the retinal lesion after their intravitreal delivery[13], and to facilitate the study of potential therapeutic interventions, such as stem cell grafts and retinal prosthesis, we subretinally injected 100 μ L of SNP solutions at three doses (0.05 mM, 0.1 mM and 0.2 mM) or NS into the posterior pole of the monkey retina next to the fovea under a surgical microscope (Figure 1A). Within 1 h after SI, SD-OCT images showed that SNP solutions or NS caused a hypo-reflective retinal bleb of about 9 mm in diameter, indicating the successful delivery (Figure 1B). In 14 cynomolgus monkeys (28 eyes) studied, 6 eyes received 0.05 mM SNP, 14 eyes 0.1 mM SNP, 5 eyes 0.2 mM SNP and 3 eyes NS, respectively. During the observation period after administration, with SD-OCT and fundus photographs, the focal lesions with various degree of severity were clearly noticed and consistently located in the posterior pole of retina in all animals including NS group. The other area of retina in the monkeys was not noticeably affected by the treatment (Figure 1C-D). All monkeys were in good health without systemic side effect or death. However, due to the unexpected injury to lens and retinal vessels in operation, complications in a few eyes

were observed, including cataract (2 eyes), vitreous hemorrhage (1), retinal tear and detachment (1), and endophthalmitis (1).

SNP caused acute retinal degeneration of cynomolgus monkeys in a dose-dependent manner

With the advantage of the noninvasive and time-saving *in vivo* measurements of retinal layers over histological test, SD-OCT was employed to dynamically evaluate the damage severity of retinal structures after the SNP treatment in the monkeys. The SI of NS or SNP solutions in three doses led to acute local retinal injury with various severities from slight, mild, moderate to severe alterations within 28 days after administration (Figure 2 A-D). In the vehicle control group, NS (3/3, 100 %) caused a slight injury with slight hyper-reflection of retinal pigment epithelium (RPE) and photoreceptor segments, sign of the cell swelling. In 0.05 mM SNP group, the majority of eyes (5/6, 83 %) presented mild injury of retina lesions with swelling, disorganization or loss of RPE, segments and part of outer nuclear layer (ONL), and 1 eye (1/6, 17 %) had severe retinal lesions with disruption of most retina and choroid. In 0.1 mM SNP group, 11 out of 14 eyes (11/14, 79 %) presented moderate injury of retina lesions with evident swelling and/or loss of RPE and outer neural retina layer, 2 out of 14 eyes (2/14, 14 %) mild and 1 out of 14 eyes (1/14, 7 %) severe. In 0.2 mM SNP group, 4 out of 5 eyes (4/5, 80 %) presented severe injury of retina lesions with the loss of most of the neural retina and RPE accompanied by the involvement of choroid, and 1 out of 5 eyes (1/5, 20 %) moderate. The time course images of SD-OCT revealed the pathological changes of cynomolgus monkey retinas over the 28-day observation period (Figure 3). The tissue swelling indicated by the hyper-reflection of retinas was marked in all SNP groups on day 7 (D7) after administration, then gradually disappeared till D28. The cell loss and disorganization of part or all of outer retinal layers were seen on D7 and thereafter in SNP treatment groups. Compared to the NS group, all of three doses of SNP caused significant reduction of the retinal thickness (from OPL to BM, and from ILM to BM) of cynomolgus monkeys over time, with the 0.2 mM SNP being the most significant (Figure 4).

Functional examination with mfERG was performed in cynomolgus monkey eyes with NS and 0.1 mM SNP SI (Figure 5 and see Additional file 1). NS SI did not cause noticeable changes in the amplitudes of P1 (Amp. P1) between pretreatment (amplitude 36.8 ± 8.1 nV/deg²) and D7 after treatment (amplitude 31.8 ± 8.2 nV/deg², $P = 0.49$; Figure 5A, C), implying the SI approach itself did not cause evident functional change of the retina. However, 0.1 mM SNP significantly reduced the responses since D7 after treatment (amplitude 13.6 ± 5.6 nV/deg², $P = 0.01$), compared to the pretreatment (amplitude 33.1 ± 5.0 nV/deg², Figure 5B-D).

SNP-induced stable and long-lasting retinal degenerations in cynomolgus monkeys

To determine the long-term effect of SNP SI on retina, some monkeys were followed up for more than 5 months after the treatment. Multimodal imaging (BAF, IR, FA and ICGA) performed in the 5th month disclosed focal lesions surrounded by relatively normal retina in the SNP groups of three doses, but no obvious damages in the NS control group. The size and shape of these damaged lesions were similar to those described above (Figure 6). Both FA and ICGA showed that fluorescence leakage and tissue

staining were obvious in the damaged area with the fluorescence intensity related to the degree of retinal damage in a concentration-dependent manner, while no leakage and tissue staining found in the control group (Figure 6).

In the 7th month after treatment, mfERG was also performed in the cynomolgus monkey eyes with 0.1 mM SNP SI. Compared to the results of the pretreatment and on the 14th days after treatment, the Amp. P1 in the 7th month were significantly reduced (see Additional file 2), indicating the SNP administration caused permanent dysfunction of retina.

Histological examination in the 7th month after SNP treatment confirmed that SNP SI caused focal retinal degeneration of cynomolgus monkeys in a dose-dependent manner, which was consistent with the results of SD-OCT described above (Figure 7). 0.05 mM SNP did not cause remarkable structural changes in retina and choroid; 0.1 mM SNP caused the depletion of outer neural layer including RPE, ONL and outer plexiform layer (OPL); while 0.2 mM SNP destroyed the entire retina and choroid. Immunostaining with the antibody recoverin revealed that photoreceptors were clearly eliminated by 0.1 mM or 0.2 mM SNP, but were almost not affected by 0.05 mM SNP.

Discussion

In the present study, a single subretinal injection of 100 μ L SNP rapidly induced a site-controllable, focal retinal degenerative lesion at the posterior pole of retina in cynomolgus monkey. The effect of SNP on retinal damage presented concentration-dependent changes. 0.1 mM SNP is the optimal dose which caused mainly depletion of outer neural retina and RPE, resembling the pathological changes of the outer retinal diseases, such as RP and AMD[1, 2]. This RD monkey model provided a valuable research platform for developing potential therapeutics, stem cell therapy in particular.

Several approaches, such as intravenous injection, intravitreal injection and SI, have been reported that could deliver retinotoxic reagents into the animal eyes to induce RD models[13]. Each approach has its own advantages and disadvantages. Intravenous injection was a common and easy method to damage the bilateral retina with large lesion areas in small and medium animals[26, 27]. However, systemic administration of retinotoxic agents would also affect the general health status of the experimental animals, and even lead to death and tumor formation[13]. Furthermore, higher dose of retinotoxic agents for large animals like monkeys was used to induce retinal damage, which caused severe renal toxicity or death[20]. Thus, intravenous injection is not suitable for large animals. On the contrary, intravitreal injection caused no severe systemic side-effects and allowed loss of the vision in only one eye, leaving a healthy control eye[28]. Nevertheless, intravitreal injection caused uneven retinal degeneration without fixed location, because the final concentration of retinotoxic agents which diffused through the vitreous and cross the entire retina to reach the photoreceptors after intravitreal injection was not even across the whole retina[21, 29]. For solving the above issues, the present study used SI to induce lesion-controllable retinal damage by delivering SNP to the target location with accurate concentration. But the operation of SI required a relatively high level of technical skills compared with other methods.

SNP, an inexpensive and clinically available drug that has been widely used to treat acute hypertension for many years, is known to release nitric oxide (NO) primarily through photochemical reactions[23, 30]. Excess NO dissolving in water can produce HNO_3 and further lead to coagulative necrosis in retina. In addition, the excessive and potent oxidant peroxynitrite (ONOO^-) rapidly produced by the interreaction of NO and oxygen free radicals also plays an important role in inducing retinal damage, such as reduced cell viability, increased cell death, leukostasis, vascular permeability and neurodegeneration[31, 32]. In addition, the enrichment in polyunsaturated lipid membranes makes the retina especially sensitive to the action of reactive oxygen and nitrogen species[32]. In our present study, SI delivered the SNP to the subretinal space and impacted the photoreceptor and RPE directly, and then reached the inner retina. Therefore, the main morphological change induced by our method was the depletion of outer neural retina and RPE, resembling the pathological changes of the outer retinal diseases, such as RP and AMD.

With the successful induction of three-dimensional (3D) retinal organoids including neural retina and RPE from hPSCs[9, 10, 33, 34], stem cell therapy, such as retinal cell transplantation, has been regarded as a potential treatment for RDs[5, 6]. Thus, carrying out the experiments *in vivo* are necessary for the safety evaluation and for the detailed analyses of efficacy, such as migration, integration or anatomy of grafts and the loss or recovery of focal retinal function. Currently, stem cell therapy might be impractical to restore or replace all the damaged cells of RD patients because of the donor cells shortage and the limitation of surgical technique[35]. In human or nonhuman primates, such as monkeys, the macula, a unique structure responsible for daytime vision and color vision, plays an important role in daily life[14]. Therefore, restoring the macular vision of RD patients is a high priority in clinical trials, which requires a suitable, focal RD monkey model. Although different approaches, such as laser photocoagulation and systemic or intravitreal delivery of retinotoxic reagents, have been tried to generate RD model in monkeys, challenges still exist in regarding to the lesion size and location, systemic side-effect and model stability[17, 20]. In this study, a focal, acute retinal degeneration lesion at the perimacular region of retina in monkeys was induced within 7 days after the SNP SI, which gradually stabilized till D28. In the 3 doses we studied, 0.1 mM SNP was proved as an optimal dose. Multimodal images and mfERG examination demonstrated there was no recovery of SNP-induced retinal degenerations in monkeys after the follow-up in more than 5 months. The above results also suggested that D28 after subretinal administration of SNP might be the befitting time for retinal cell transplantation. Hence, this lesion-controllable monocular model is not only ethically preferable, but also allows longer monitoring and detailed analyses for stem cell therapy.

This study also has some limitations. Compared with intravitreal injection, the operation of subretinal injection is more complicated and difficult. Some complications in eyes occurred. In addition, compared with transgenic models, the damage range is still not large enough.

Conclusion

This study established a rapid and lesion-controllable RD monkey model by subretinal injection of SNP without severe systemic side-effects, and founded that 0.1 mM was the optimal dose, which provides an

improved tool for the development of new therapies, such as stem cell therapy, for RD.

Abbreviations

RD

Retinal degeneration; AMD:Age-related macular degeneration; RP:Retinitis pigmentosa; RPE:Retinal pigment epithelium; hPSC:Human pluripotent stem cell; MNU:N-methyl-N-nitrosourea; SI:Subretinal injection; SNP:Sodium nitroprusside; NS:Normal saline; SD-OCT:Spectral-domain optical coherence tomography; BAF:BluePeak-autofluorescence; cSLO:Confocal scanning laser ophthalmoscope; FA:Fluorescein angiography; ICGA:Indocyanine green angiography; H&E:Hematoxylin and eosin; MfERG:multifocal electroretinogram; ONL:Outer nuclear layer; OPL:Outer plexiform layer; ORL:outer retinal layer; ILM:internal limiting membrane; BM:Bruch' membrane; IR:Infrared Radiation; NO:Nitric oxide; 3D:three-dimensional.

Declarations

Ethics approval and consent to participate

The study design and experimental protocols were approved by the Application Format of Animal Experimental Ethical Inspection to Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-Sen University (no. 2016-012 and 2019-042). All experimental procedures involving animals adhered to the Association Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

Consent for publication

Not applicable.

Availability of data and materials

Please contact the author for data requests.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by grants from the Science & Technology Project of Guangdong Province (2017B020230003), the National Key R&D Program of China (2017YFA0104100; 2016YFC1101103), the National Natural Science Foundation of China (81970842, 81570874), and the Science & Technology Project of Guangzhou (201803010078).

Authors' contributions

G.G. and L.H. were responsible for the collection and/or assembly of data, data analysis and interpretation, and manuscript writing. S.L. was responsible for the collection and/or assembly of data. D.Z. and X.S. performed the SD-OCT; W.Z. performed the H&E staining. M. Y. and G. L. guided the operation of mfERG and data interpretation. X.Z. was responsible for the conception and design, data analysis and interpretation, manuscript writing and proof, administrative and financial support. All authors read and approved the final manuscript.

Acknowledgments

None.

References

1. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *Lancet*. 2018;392:1147–59.
2. Verbakel SK, van Huet RAC, Boon CJF, den Hollander AI, Collin RWJ, Klaver CCW, et al. Non-syndromic retinitis pigmentosa. *Prog Retin Eye Res*. 2018;66:157–86.
3. Brito-Garcia N, Del Pino-Sedeno T, Trujillo-Martin MM, Coco RM, Rodriguez de la Rúa E, Del Cura-Gonzalez I, et al. Effectiveness and safety of nutritional supplements in the treatment of hereditary retinal dystrophies: a systematic review. *Eye (Lond)*. 2017;31:273–85.
4. Cheng DL, Greenberg PB, Borton DA. Advances in Retinal Prosthetic Research: A Systematic Review of Engineering and Clinical Characteristics of Current Prosthetic Initiatives. *Curr Eye Res*. 2017;42:334–47.
5. Llonch S, Carido M, Ader M. Organoid technology for retinal repair. *Dev Biol*. 2018;433:132–43.
6. Gagliardi G, Ben M'Barek K, Goureau O. Photoreceptor cell replacement in macular degeneration and retinitis pigmentosa: A pluripotent stem cell-based approach. *Prog Retin Eye Res*. 2019;71:1–25.
7. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;282:1145–7.
8. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861–72.
9. Nakano T, Ando S, Takata N, Kawada M, Muguruma K, Sekiguchi K, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell*. 2012;10:771–85.
10. Zhong X, Gutierrez C, Xue T, Hampton C, Vergara MN, Cao LH, et al. Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. *Nat Commun*. 2014;5:4047.
11. Li G, Xie B, He L, Zhou T, Gao G, Liu S, et al. Generation of Retinal Organoids with Mature Rods and Cones from Urine-Derived Human Induced Pluripotent Stem Cells. *Stem Cells Int* 2018; 2018:4968658.
12. Liu Y, Xu HW, Wang L, Li SY, Zhao CJ, Hao J, et al. Human embryonic stem cell-derived retinal pigment epithelium transplants as a potential treatment for wet age-related macular degeneration.

Cell Discov. 2018;4:50.

13. Lin TC, Seiler MJ, Zhu D, Falabella P, Hinton DR, Clegg DO, et al. Assessment of Safety and Functional Efficacy of Stem Cell-Based Therapeutic Approaches Using Retinal Degenerative Animal Models. *Stem Cells Int* 2017; 2017:9428176.
14. Shibuya K, Tomohiro M, Sasaki S, Otake S. Characteristics of structures and lesions of the eye in laboratory animals used in toxicity studies. *J Toxicol Pathol.* 2015;28:181–8.
15. Liang L, Katagiri Y, Franco LM, Yamauchi Y, Enzmann V, Kaplan HJ, et al. Long-term cellular and regional specificity of the photoreceptor toxin, iodoacetic acid (IAA), in the rabbit retina. *Vis Neurosci.* 2008;25:167–77.
16. Scott PA, Kaplan HJ, Sandell JH. Anatomical evidence of photoreceptor degeneration induced by iodoacetic acid in the porcine eye. *Exp Eye Res.* 2011;93:513–27.
17. Shirai H, Mandai M, Matsushita K, Kuwahara A, Yonemura S, Nakano T, et al. Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *Proc Natl Acad Sci U S A.* 2016;113:E81–90.
18. Organisciak DT, Vaughan DK. Retinal light damage: mechanisms and protection. *Prog Retin Eye Res.* 2010;29:113–34.
19. Iseli HP, Korber N, Karl A, Koch C, Schuldt C, Penk A, et al. Damage threshold in adult rabbit eyes after scleral cross-linking by riboflavin/blue light application. *Exp Eye Res.* 2015;139:37–47.
20. Ou Q, Zhu T, Li P, Li Z, Wang L, Lian C, et al. Establishment of Retinal Degeneration Model in Rat and Monkey by Intravitreal Injection of Sodium Iodate. *Curr Mol Med.* 2018;18:352–64.
21. Rosch S, Werner C, Muller F, Walter P. Photoreceptor degeneration by intravitreal injection of N-methyl-N-nitrosourea (MNU) in rabbits: a pilot study. *Graefes Arch Clin Exp Ophthalmol.* 2017;255:317–31.
22. Enzmann V, Row BW, Yamauchi Y, Kheirandish L, Gozal D, Kaplan HJ, et al. Behavioral and anatomical abnormalities in a sodium iodate-induced model of retinal pigment epithelium degeneration. *Exp Eye Res.* 2006;82:441–8.
23. Isago H, Sugano E, Murayama N, Tamai M, Tomita H. Establishment of monocular-limited photoreceptor degeneration models in rabbits. *BMC Ophthalmol.* 2013;13:19.
24. Li K, Liu S, Zhong X, Ge J. Generation of an acute retinal photoreceptor degeneration model in rabbits. *Am J Transl Res.* 2018;10:235–45.
25. Hood DC, Bach M, Brigell M, Keating D, Kondo M, Lyons JS, et al. ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition). *Doc Ophthalmol.* 2012;124:1–13.
26. Redfern WS, Storey S, Tse K, Hussain Q, Maung KP, Valentin JP, et al. Evaluation of a convenient method of assessing rodent visual function in safety pharmacology studies: effects of sodium iodate on visual acuity and retinal morphology in albino and pigmented rats and mice. *J Pharmacol Toxicol Methods.* 2011;63:102–14.
27. Chen YY, Liu SL, Hu DP, Xing YQ, Shen Y. N -methyl- N -nitrosourea-induced retinal degeneration in mice. *Exp Eye Res.* 2014;121:102–13.

28. Cho BJ, Seo JM, Yu HG, Chung H. Monocular retinal degeneration induced by intravitreal injection of sodium iodate in rabbit eyes. *Jpn J Ophthalmol*. 2016;60:226–37.
29. Hara A, Niwa M, Aoki H, Kumada M, Kunisada T, Oyama T, et al. A new model of retinal photoreceptor cell degeneration induced by a chemical hypoxia-mimicking agent, cobalt chloride. *Brain Res*. 2006;1109:192–200.
30. Siu AW, Ortiz GG, Benitez-King G, To CH, Reiter RJ. Effects of melatonin on the nitric oxide treated retina. *Br J Ophthalmol*. 2004;88:1078–81.
31. Goldstein IM, Ostwald P, Roth S. Nitric oxide: a review of its role in retinal function and disease. *Vision Res*. 1996;36:2979–94.
32. Opatrilova R, Kubatka P, Caprnda M, Busselberg D, Krasnik V, Vesely P, et al. Nitric oxide in the pathophysiology of retinopathy: evidences from preclinical and clinical researches. *Acta Ophthalmol*. 2018;96:222–31.
33. Liu S, Xie B, Song X, Zheng D, He L, Li G, et al. Self-Formation of RPE Spheroids Facilitates Enrichment and Expansion of hiPSC-Derived RPE Generated on Retinal Organoid Induction Platform. *Invest Ophthalmol Vis Sci*. 2018;59:5659–69.
34. Luo Z, Zhong X, Li K, Xie B, Liu Y, Ye M, et al. An Optimized System for Effective Derivation of Three-Dimensional Retinal Tissue via Wnt Signaling Regulation. *Stem Cells*. 2018;36:1709–22.
35. Gasparini SJ, Llonch S, Borsch O, Ader M. Transplantation of photoreceptors into the degenerative retina: Current state and future perspectives. *Prog Retin Eye Res*. 2019;69:1–37.

Figures

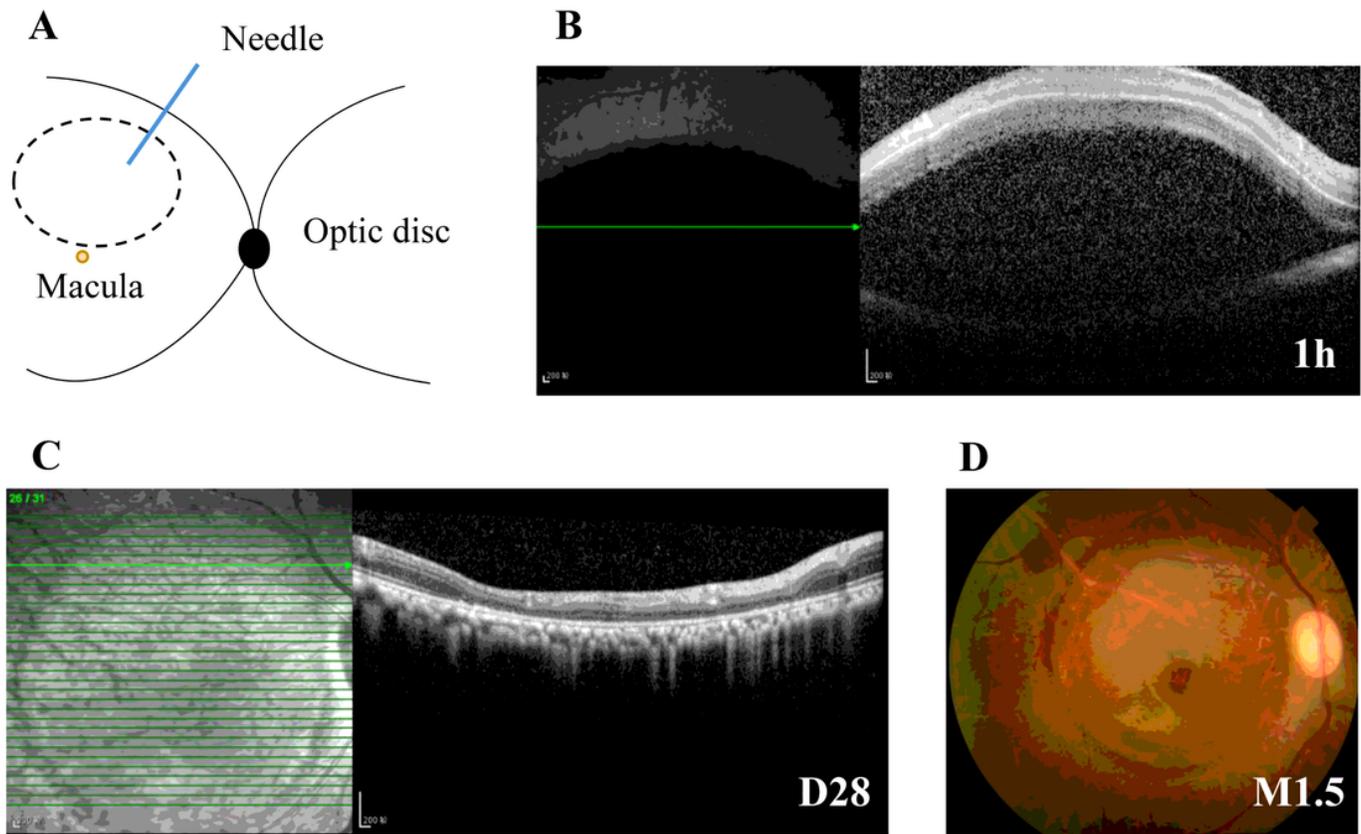


Figure 1

The subretinal injection (SI) of SNP caused focal retinal lesion in monkeys. A. Illustration depicts the SI site at the posterior pole of retina. B. A typical SD-OCT image confirming the formation of retinal bleb 1 h after SI which indicated the successful delivery of SNP solution. C. The typical SD-OCT image shows the focal retinal degeneration induced by SNP SI on D28 after the SI. D. Fundus photograph also showed the local lesion surrounded by the healthy retina 1.5 months after the SI.

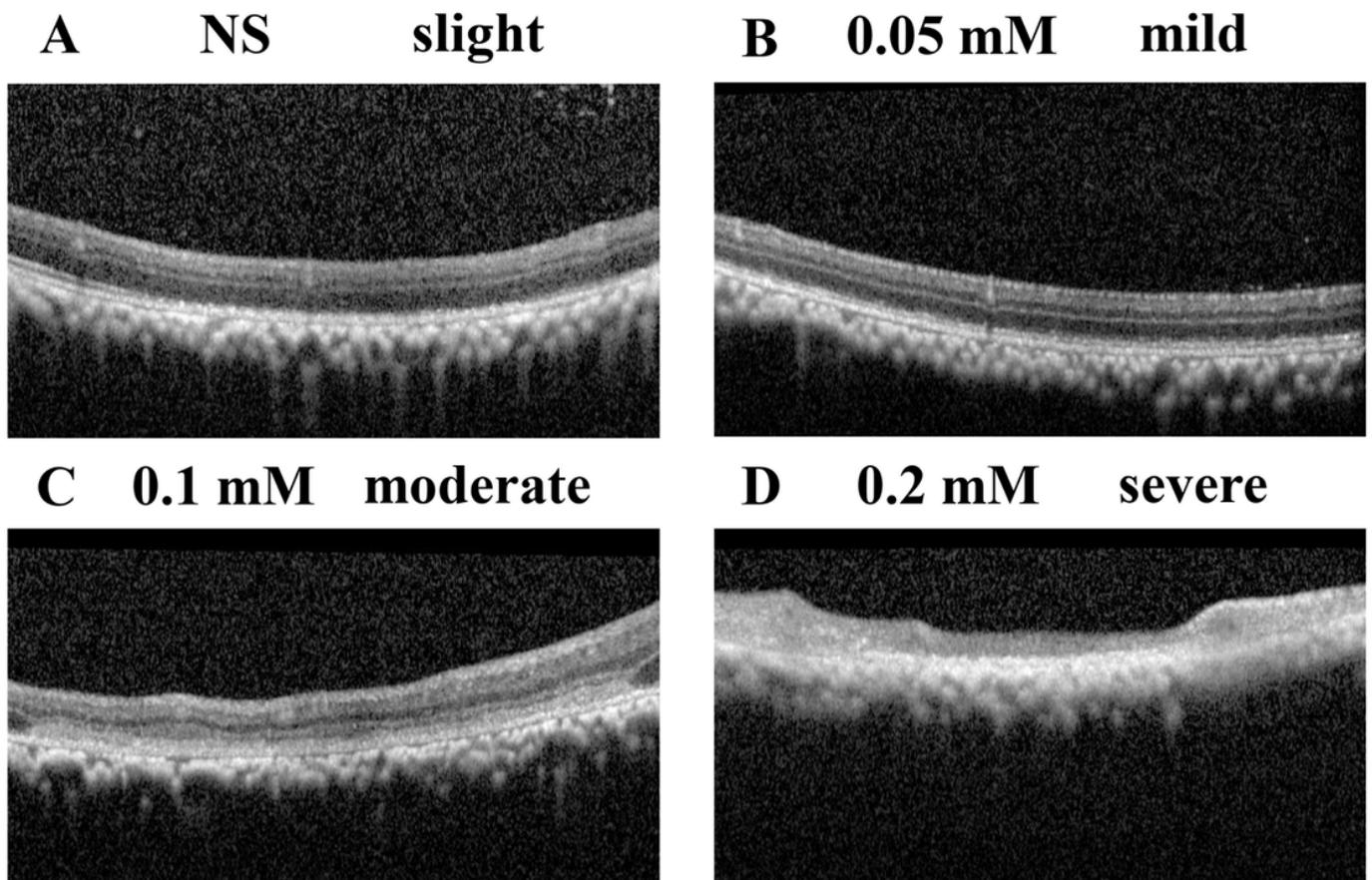


Figure 2

SNP administration induced retinal degeneration in monkeys in dose-dependent manner. A-D. Typical SD-OCT images indicate the severity and lesion size of NS and SNP in three doses on D7 after SI. A. NS caused a slight lesion with the high reflection of photoreceptor segments and the unsmooth reflection of RPE. B. 0.05 mM led to a mild damage similar to the NS group. C. 0.1 mM caused a moderate damage with hyper-reflective signals of ONL and RPE. D. 0.2 mM caused an acute, severe damage with significantly swelling, thinned retina and choroid. ONL: outer nuclear layer.

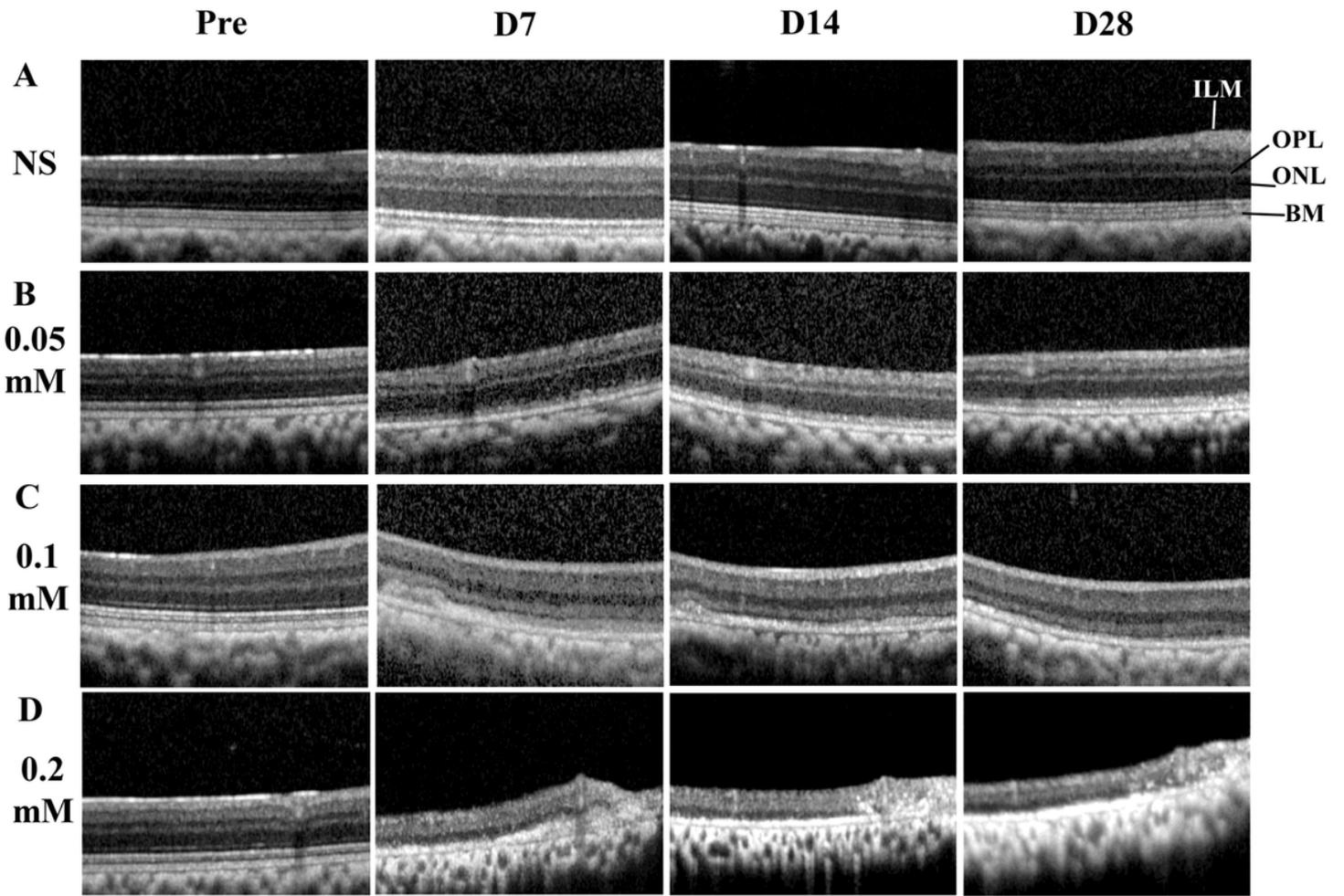


Figure 3

SD-OCT images showing the dynamic changes of retinal structures within 28 days after SNP treatment. A. NS, B. 0.05 mM SNP, C. 0.1 mM SNP, D. 0.2 mM SNP.

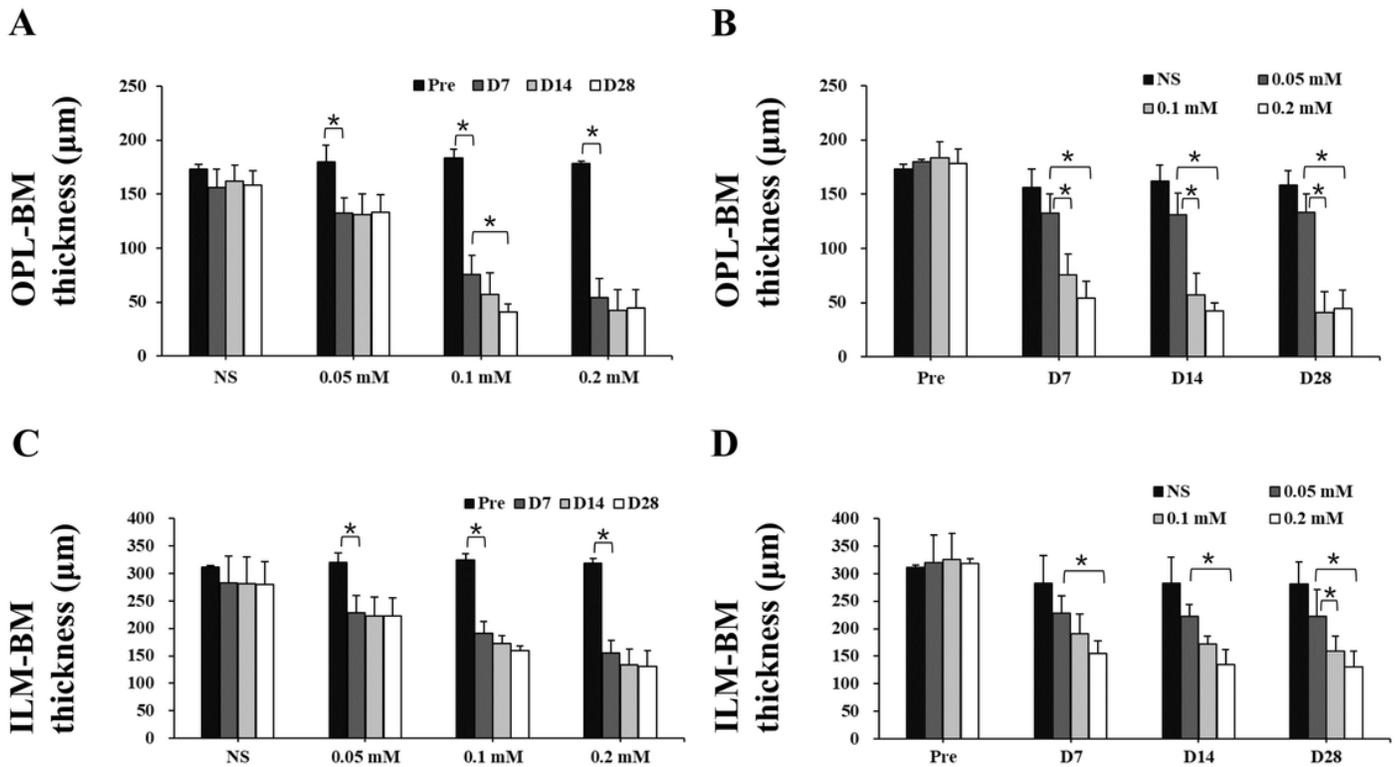


Figure 4

Retinal thickness of monkeys decreased after SNP treatment in both dose- and time-dependent manner. A-B. The thickness ORL from OPL to BM was significantly decreased in 28 days after SNP SI in time-dependent (A) and dose-dependent manner (B). C-D. The thickness of the whole retina from ILM to BM was significantly decreased in 28 days after SNP SI in time-dependent (C) and dose-dependent manners (D). $P < 0.05$. ORL: Outer retinal layer; ILM: Internal limiting membrane; OPL: Outer plexiform layer; BM: Bruch' membrane.



Figure 5

MfERG showed a significant reduction in retinal response of SNP-damaged area. A-B. Typical mfERG results of NS and 0.1 mM SNP treatments on D7 after SI. C-D. The averaged responses of P1 in NS group and 0.1 mM SNP group in an observation period of 7 days and 28 days, respectively. \boxtimes : $P < 0.05$.

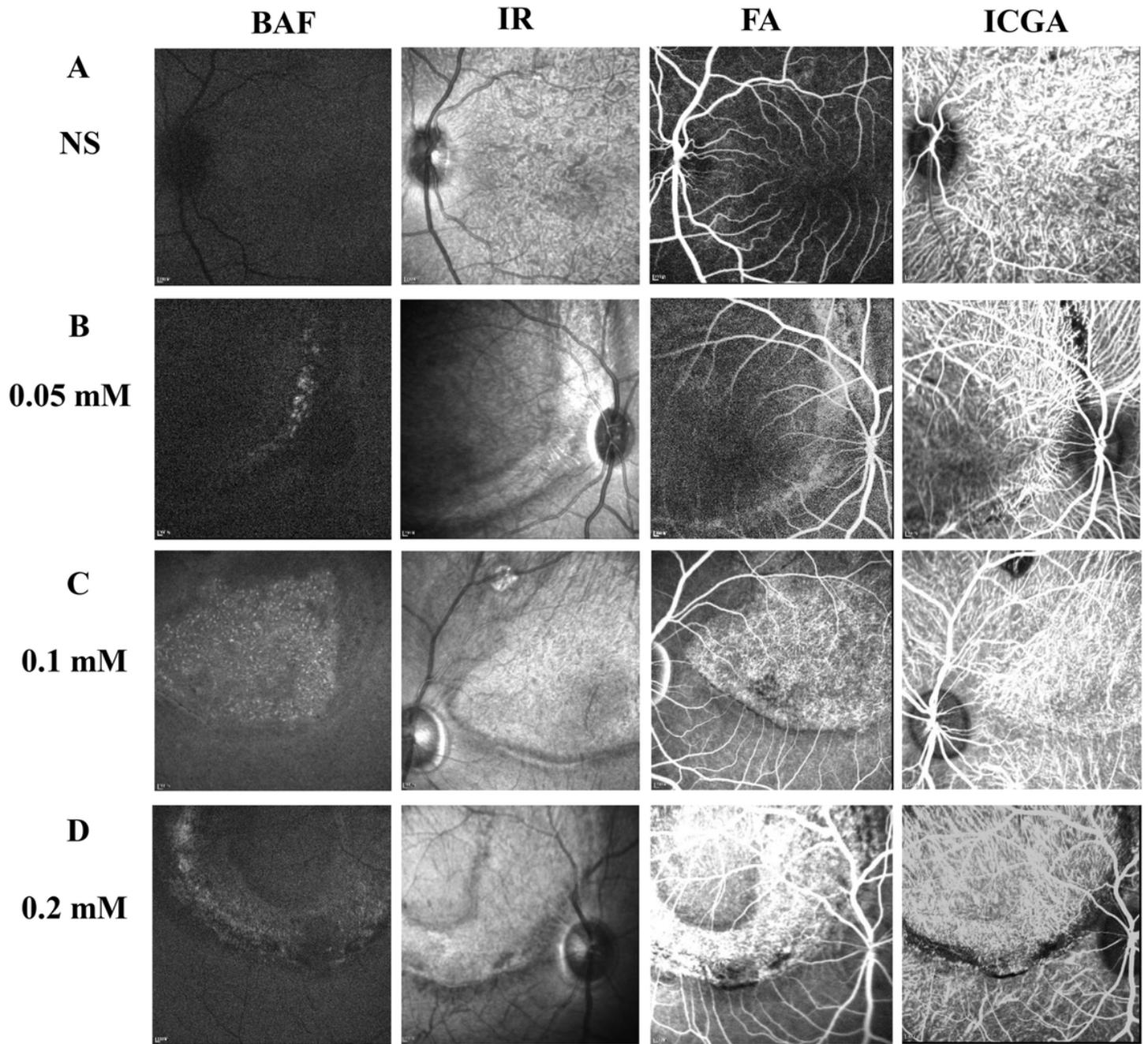


Figure 6

No recovery of SNP-induced retinal damages shown in multimodal images after long-term follow-up. A-D. Multimodal imaging of BAF, IR, FA and ICGA performed in the 5th month in the SNP groups of three doses and the NS control group. BAF: BluePeak-autofluorescence; IR: Infrared Radiation; FA: Fluorescein angiography; ICGA: Indocyanine green angiography.

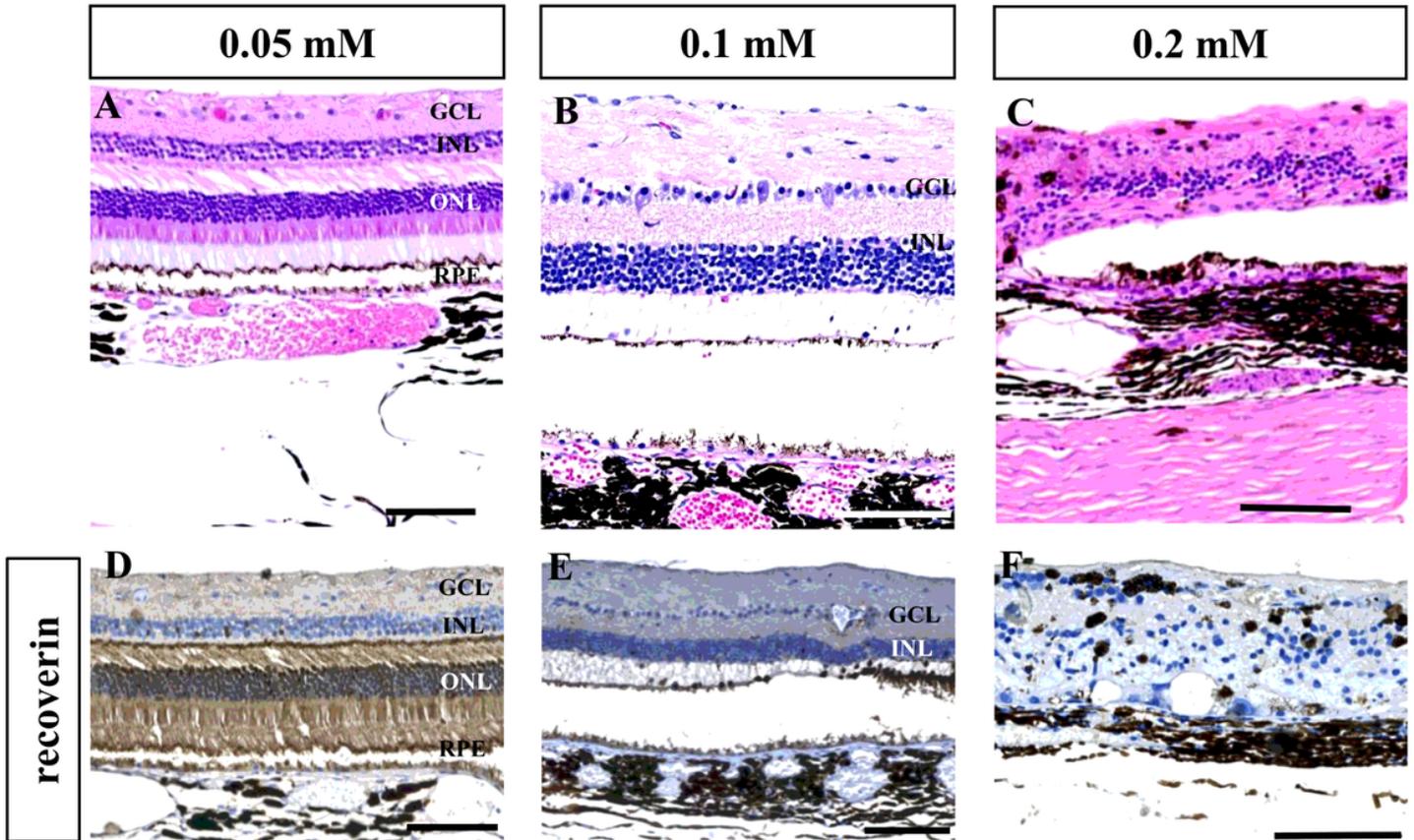


Figure 7

H&E and immunofluorescence staining of the retina in monkeys in 7th month after SNP SI. A-C. H&E staining in the SNP groups with 0.05, 0.1 and 0.2 mM doses, respectively. D-E. Recoverin positive photoreceptor cells presented normally in 0.05 mM group, almost all disappeared in 0.1 mM group, and completely disappeared in 0.2 mM groups. Scale bar: 100 μ M.

Supplementary Files

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

- [Supplementaryinformation.docx](#)
- [Supplementaryinformation.docx](#)
- [manuscript20200813final.pdf](#)
- [manuscript20200813final.pdf](#)
- [FigS2.tif](#)
- [FigS2.tif](#)
- [FigS1.tif](#)
- [FigS1.tif](#)