May 13, 2024

Protocol for development and temporal in vivo imaging of RVO mouse models

DOI
dx.doi.org/10.17504/protocols.io.ewov19nrplr2/v1

Xu Xiaowei[1]

[1]Department of Ophthalmology and Research Laboratory of Macular Disease, West China Hospital, Sichuan University, Chengdu, Sichuan, China.

Xu Xiaowei
Department of Ophthalmology and Research Laboratory of Macul...

DOI: https://dx.doi.org/10.17504/protocols.io.ewov19nrplr2/v1

Protocol Citation: Xu Xiaowei 2024. Protocol for development and temporal in vivo imaging of RVO mouse models. **protocols.io https://dx.doi.org/10.17504/protocols.io.ewov19nrplr2/v1**

Protocol status: Working
We use this protocol and it's working

Created: May 12, 2024

Last Modified: May 13, 2024

Protocol Integer ID: 99621

Keywords: rvo mouse model, vivo imaging, temporal in vivo imaging, imaging

Abstract

This is an protocol for development and temporal in vivo imaging of RVO mouse models.

Troubleshooting

Animals and Anesthesia

1 Male *C57BL/6J* mice, aged 6-8 weeks and weighing 19-21g, were purchased from DOSSY EXPERIMENTAL ANIMALS CO. LTD, Chengdu, China. The mice were adaptively raised in an environment with a 12h/12h light/dark cycle, constant room temperature of 26°C, and adequate water and food for one week before the experiment. All experimental procedures followed the Statement for the Use of Animals in Ophthalmic and Vision Research of the Association for Research in Vision and Ophthalmology (ARVO) and were under the supervision of the Institutional Animal Care and Use Committee (IACUC) of Sichuan University (Protocol Number: 20220907001). Before RVO modeling and imaging examination, mice were routinely anesthetized by intraperitoneal injection of 1% sodium pentobarbital, 0.15mL/20g, and pupils were dilated by Mydrin® eyedrops (Santen Pharmaceutical Co., LTD., Osaka, Japan). The mice were euthanized by cervical dislocation at the end of the intervention.

Laser photocoagulation for the RVO mouse model

2 The RVO mouse model was generated using a slit lamp system comprising a slit lamp microscope (66 Vision Tech Co.,Ltd. Suzhou, China) and a Nd:GdVO4 532nm laser photocoagulator (MEDA Co.,Ltd. Tianjin, China). Instrument pre-adjustments included maximizing slit width, setting a 0° light source angle, adjusting microscope magnification to 40X with the focal point aligned with the laser photocoagulator, and placing the laser indicator at the center of the slit lamp illumination. After pupil dilation, slit lamp microscopy was conducted to exclude individuals with refractive media opacity or fundus deformity. Mice were then anesthetized immediately following a tail vein injection of the photosensitizer Rose Bengal (40mg/kg, 8mg/mL). A cover glass coated with viscoelastic agents was applied to flatten the cornea and ensure visibility of the fundus structure. 15 minutes was ensured for photosensitizer systematic circulation when a state of surgical anesthesia was achieved before the laser procedure.

3 Acute RVO mouse model: The laser parameters settings were powers of 80mW, 100mW, and 120mW, duration of 1s, and spot size of 50μm. 4 laser shots were performed continuously at the same location 2 to 3 papillary diameters (PD) away from the optic disc for one major vein, and a total of 3 veins for each eye. When only one or no vein was occluded, an additional vein was photocoagulated with a total number of no more than four.

4 Chronic RVO mouse model: The laser parameters settings were power of 120mW, duration of 1s, and spot size of 50μm. Consecutive 10 to 15 laser shots for a target vein started from 3PD from the optic disc towards the proximal end with an occlusion length of 1.5 to 2PD.

5 Sham laser group: As a control for the acute RVO mouse model, no photosensitizer, identical laser parameters, and 4 continuous laser shots were performed 2 to 3PD from the optic disc avoiding large retinal vessels at each of three sites per eye.

6 Double-eye modeling was conducted on approximately 10 experimental animals to ensure that each group had 20 to 30 veins successfully occluded after photocoagulation.

Scanning laser ophthalmoscopy (SLO)

7 SLO was performed using Heidelberg Spectralis HRA+OCT Fundus Diagnostic Instrument (Heidelberg Engineering, Inc.) with a widefield lens for an en-face view of the normal and RVO retinal morphology. The Multicolor (Mcolor) and Infrared (IR) examination modes (55°×55° field; high-resolution scan mode; automatic real-time mean (ART) up to 100 frames) were applied for the acute model and sham laser group immediately after photocoagulation (day 0) and on the day of 1, 2, 3, 5 and 7 after RVO. The 80mW, 100mW, and 120mW groups were followed up with the same method immediately and on days 1 and 7 after RVO. During the evaluation of the anti-VEGF treatment efficacy in the acute model, IR was performed on days 1 and 2 after RVO. The chronic model only required an IR test immediately and on days 7, 14, 21, and 31 after RVO.

Fundus fluorescein angiography (FFA)

8 FFA was implemented through Heidelberg Spectralis HRA+OCT with a widefield lens in fluorescein angiography (FA) mode (55°×55° field; high-resolution scan mode; ART up to 100 frames). Mice were injected intraperitoneally with 0.2 mL of 2% sodium fluorescein after pupil dilation and anesthesia. Venous obstruction was selectively examined 2 min after the injection in the acute model groups, at the time points when the results of SLO were ambiguous. In the chronic model group, venous occlusion and retinal perfusion at days 7, 14, 21, and 31 after RVO were evaluated with no strict requirement for imaging time after contrast agent injection, as the animal posture was needed to constantly adjust for the peripheral retina entering the field of view. Normal posterior pole and peripheral fundus FA imaging were also recorded.

Optical coherence tomography (OCT)

9 OCT images of the retinal transverse view were promptly captured for the acute RVO model and the sham laser group post-photocoagulation and at 1, 2, 3, 5, and 7 days after RVO. Normal eyes were also imaged for reference. Additionally, OCT images for the 80mW, 100mW, and 120mW groups were acquired immediately after photocoagulation, as well as at 1 and 7 days after RVO. In accessing the responsiveness to anti-VEGF medications in the acute model, OCT scans were performed post-photocoagulation, and

on days 1 and 2 after RVO. After the mice were anesthetized with the pupil sufficiently dilated, Heidelberg Spectralis HRA+OCT entered the IR+OCT combined imaging mode with a standard lens (30°×30° field; high-speed scan mode; ART up to 100 frames). OCT images were collected at the distal tangent of the laser spot after RVO and 3PD off the optic disc in normal eyes as the baseline guided by IR. The option of follow-up was checked during the observation window to ensure that the examination was performed at the same site. OCT images were quantitatively analyzed using Heidelberg Eye Explorer software for retinal layer thickness assessment, with manual correction for retinal delamination results. Follow-up time points with refractive media opacity or severe retinal detachment (RD) were excluded.

Histology and immunofluorescence

10 Histology and immunofluorescence were performed on normal eyes of mice to clarify the retinal layers in the longitudinal section and the distribution of retinal capillaries, respectively.

11 Histology: The mice were euthanized, and the eyeballs were enucleated and fixed in 4% paraformaldehyde. After routine dehydration, the eyeballs were embedded in paraffin, cut into 5 μm slices, and stained with hematoxylin-eosin for observation.

12 Immunofluorescence: Eyeballs were enucleated and fixed as before, embedded in optimal cutting temperature compound, frozen, and cut into 10μm slices at -20°C. After incubation with isolectin B4 (1:200, Sigma-Aldrich, St Louis, MO, United States) at 4°C overnight and stained with 4',6-diamidino-2-phenylindole (DAPI), the slices were observed under the fluorescence microscope (Carl Zeiss, Germany).

Drug administration

13 Immediately after acute RVO model induction, experimental animals received drug administration via intravitreal injection. A 30-gauge needle punctured 1mm posterior to the nasal limbus, followed by a 34-gauge microsyringe (Hamilton (shanghai) Laboratory Equipment Co., Ltd) inserted vertically and advanced approximately 60° towards the optic nerve in the vitreous cavity for about 1mm, after which the drug was slowly and steadily injected. 14 eyes in the experimental group received Aflibercept (Eylea®, Bayer AG) at 10 μg/μL, 2μL per eye; 12 eyes in the control group were given an equal amount of phosphate-buffered saline (PBS).

Statistical analysis

14 Data were presented as mean±SEM, rate, and percentage. The Chi-square test (followed by Bonferroni multiple comparisons) was employed to compare the rates and percentages among two (or more) groups. The comparison between two groups of samples conforming to normality was conducted by Student's t-test, with Welch

correction applied in case of inequality of variance. The Mann-Whitney U test was employed for datasets not adhering to normality. A P-value of less than 0.05 was deemed to signify statistical significance. The statistical analysis was completed with the software SPSS 25.0 (SPSS, Inc. Chicago, IL, United States).