An Experimental Proliferative Vitreoretinopathy (PVR) Model in Pigmented Rabbits for Testing of New Treatment

Lichun Zhong and Laxman S. Desai, Ocular Science Department, Toxikon Corporation, Bedford, Massachusetts 01730, USA

ABSTRACT

Purpose: To create an experimental proliferative vitreoretinopathy (PVR) model induced by injecting rabbit conjunctival fibroblast cells (RCFs) into the vitreous cavity for testing the efficacy of potential compounds to discover new treatments for patients with PVR.

Method: Seventeen (17) New Zealand red pigmented rabbits were used. One (1) of the rabbits was used to generate RCFs. Both conjunctiva were dissected, resectioned, and planted in 12-well plate for primary tissue culture. Cells from the primary tissue culture were cultured for 3-5 passages to generate RCFs. After four (4) – five (5) weeks, the concentration of the RCFs was 5.0 x 10^6/100μL of balance salt solution (BSS) and the RCF viability was identified by Trypan Blue Test. Eight (8) male and eight (8) female pigmented rabbits were used to create the PVR model. After the animals were sedated systemically with ketamine (35 mg/kg) and xylazine (5 mg/kg) and their eyes were topically anesthetized with 0.5% proparacaine, one eye of each rabbit was injected once with 5.0 x 10^5 RCFs in 100μL of BSS into the vitreous body. The day of the RCF injection was designated as Day 1 and the last day of the study was Day 28. The eye of the RCF injection was designated as the PVR eye and the contralateral eye as the non-PVR eye. After the pupils were dilated with a 1% tropicamide ophthalmic solution, all animals received oculaur examinations (OE) for signs of PVR at pre-study, Day 1, Day 3, weekly, and at pre-sacrifice using a slit lamp biomicroscope and a surgical microscope with a glass slide or an indirect ophthalmoscope. Classification of the PVR is divided into six (6) stages (0-5) using the clinical criteria. Intraocular Pressure (IOP) measurement: IOPs of all animals were measured. Ocular Examinations (OE): All eyes of animals were examined and the PVR eyes were scored for signs of the PVR. Classification of the PVR was divided into six (6) stages using the clinical criteria described by Fastenberg et al. (See Criteria for Stages of PVR below).

RESULTS

Study Design:

Animals: Nine (9) male and eight (8) female New Zealand red pigmented rabbits.

RCF Preparation: One male rabbit was sacrificed five (5) weeks before the RCF injection. Both conjunctiva were dissected, resectioned, and cultured with DMEM medium for 3-5 passages to generate 5.0 x 10^6 (half millions) of RCFs per eye.

Induction of PVR: One eye of each animal was injected with 5.0 x 10^5 (half millions) of RCFs in 100 μL balanced salt solution (BSS) into the vitreous body once on Day 1 following 4 weeks.

Intraocular Pressure (IOP) Measurement: IOPs of all animals were measured. Ocular Examinations (OE): All eyes of animals were examined and the PVR eyes were scored for signs of the PVR.

CONCLUSIONS

The PVR model was successfully induced and demonstrated 28 days after one injection of RCFs. The PVR model provides a stable, effective and reliable method for testing and development of new treatments for patients with PVR.

METHODS AND MATERIAL

Criteria for Stages of PVR:

Stage Characteristics
0 Normal eye
1 Intravitreal membrane
2 Focal traction, Localized vascular changes, Hyphema, engorgement, dilation, Blood vessel elevation
3 Localized detachment of mediay ray
4 Extensive retinal detachment, Total medullary ray detachment, Peripapillary retinal detachment
5 Total retinal detachment, Retinal folds and holes

RCF Culture (Magnification x 40):

Rabbit Conjunctival Culture RCF Passage 1 RCF Passage 3

PVR Stage (Grades 0-5):

PVR Stage (Images):

PVR in the Vitreous Cavity (Day 28):

Magnification x 40 x 200 x 40 x 200