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

Purpose: The purpose of the study was 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.

Methods: Seventeen (17) New Zealand Red pigmented rabbits were used in the study. One (1) of rabbits was used to generate RCFs. Both conjunctiva were dissected, resectioned, and placed 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) week RCF culture, the concentration of the RCF was 5.0 x 10^5 /100ul of balanced 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. One eye of each rabbit was injected with 5.0 x 10^5 RCFs in 100 µL BSS into the vitreous body once after animals were sedated systemically with ketamine (35 mg/kg) and xylazine (5 mg/kg) and eyes were topically anesthetized with 0.5% proparacaine. 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. All animals were examined ocular 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 after pupils were dilated with 1% tropicamide ophthalmic solution. Classification of the PVR is divided into six (6) stages (0-5) using the clinical criteria. Intraocular Pressure (IOP) in all animals was measured at pre-study, Day 1, Day 3, weekly and at pre-sacrifice using a TonoVet. Ocular Pressure (OP) was performed for the posterior segments and retinas in animals and the signs of the PVR were photographed at pre-study, Day 1, Day 3, weekly and at pre-sacrifice. Animals were weighed at pre-study, weekly and at pre-sacrifice. Animals were observed for clinical observation (CO) daily and morbidity/mortality (MM) twice daily. Animals were monitored daily for signs of distress and any additional gross ocular observations were recorded. Animals were injected intravenously with Euthasol (120 mg/kg) at the end of the study. At sacrifice, all eyes were enucleated and frozen at ~80 °C for three (3) days and then fixed in Modified Davidson’s fixative medium 18-24 hours. The eyeball were shifted to alcohol for 50% (to (6) – eight (8) hours and 70% for 12-16 hours and then removed to 100% neutral and buffered formalin to be ready for being paraffin-embedded and sectioned with five (5) – six (6) µm thick. All slides will be stained with hematoxylin and eosin using standard techniques for pathological evaluation.

Results: After RCF injection, 16 animals exhibited the PVR in the vitreous body of the PVR eyes. The PVR of eight (8) animals reached Stage 4 and eight (8) animals reached Stage 5 by Day 28. All vitreous bodies in the PVR eyes of all animals were scored 0 as normal. IOP measurements in the PVR eyes showed no difference compared to those in the non-PVR eyes (p<0.05). None of the animals had any change their body weight and no significant clinical signs were observed during the course of the study. Histopathological analysis showed that fibrosis/fibroblast cells were noted and scored minimal to moderate in vitreous body and/or choroid and/or retinal/optic disc in all animals and retinal detachments were found in 11 animals in the PVR eyes, based on the microscopic observations.

Conclusions: The PVR model was 100% successfully induced and demonstrated 28 days after one injection of RCFs in 16 New Zealand Red pigmented rabbits. The pigmented rabbit PVR model dose provide a stable, effective and reliable method for testing and development of new treatments for patients with PVR.

METHODS AND MATERIAL

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, re-sectioned, and cultured with DMEM medium for 3-5 passages to generate 5.0 x 10^5 (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.

Intercular 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 Table below).

Criteria for Stages of PVR

<table>
<thead>
<tr>
<th>Stage</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal eye</td>
</tr>
<tr>
<td>1</td>
<td>Intravitreal membrane</td>
</tr>
<tr>
<td>2</td>
<td>Focal traction, Localized vascular changes, Hyhema, engorgement, dilation, Blood vessel elevation</td>
</tr>
<tr>
<td>3</td>
<td>Localized detachment of medullary ray</td>
</tr>
<tr>
<td>4</td>
<td>Extensive retinal detachment, Total medullary ray detachment, Peripapillary retinal detachment</td>
</tr>
<tr>
<td>5</td>
<td>Total retinal detachment, Retinal folds and holes</td>
</tr>
</tbody>
</table>

RCF Culture (Magnification x 40)

Rabbit conjunctival culture RCF Passage 1 RCF Passage 3

RESULTS

IOP Measurement:

PVR Stage (Graph):

PVR Stage (Images):

Stage 1 (Day 7) Stage 2 (Day 14) Stage 3 (Day 21) Stage 4 (Day 28) Stage 5 (Day 35) Stage 6 (Day 42)

Time after RCF injection (Day):


PVR Stage (Images): Day 1 Day 2 Day 3 Day 4 Day 5 Day 6

PVR in the Vitreous Cavity (Day 28)

Left eye (Control) Right eye (PVR model)

Magnification x 40 x 200 x 40 x 200

CONCLUSIONS

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