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A new model of experimental subretinal neovascularization in the rabbit

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Abstract

Existing animal models of choroidal neovascularization (CNV) present several problems: they are hard to reproduce, they are inefficient, and the CNV created is not sustainable. The purpose of this study is to develop a highly efficient, reliable, sustainable rabbit model of CNV to facilitate the study of anti-angiogenic and anti-proliferative therapies for ocular diseases. Twenty-two pigmented rabbits were used in this study. Eleven rabbits received subretinal injections of either 10 μl of Matrigel with 500 ng of vascular endothelial growth factor (VEGF) or 20 μl of Matrigel with 750 ng of VEGF; eight rabbits received subretinal injections of either 10 or 20 μl of Matrigel only; three rabbits used as controls received subretinal injections of 20 μl phosphate-buffered saline (PBS) alone. Fundus photography, fluorescein angiography, optical coherence tomography, and histologic examinations were performed 1, 2, 4, and 9 weeks after injection. All experimental eyes showed angiographic leakage within this localized area 1 week after injection. The amount of leakage usually increased at weeks 2 and 4 and, in most cases, persisted at week 9. Control eyes demonstrated no leakage at any time point. Optical coherence tomography of treated eyes showed subretinal fluid and the presence of a lesion, possibly vascular or fibrotic, at the site of the leakage. Histologic analysis confirmed the presence of new subretinal blood vessels in the areas of Matrigel deposit. In conclusion, this novel method provides a highly reproducible, reliable, and sustainable rabbit model of experimental choroidal neovascularization. Such a model may prove useful for screening new anti-angiogenic therapies in a larger animal eye.
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1. Introduction

Choroidal neovascularization (CNV) has long been recognized as a clinicopathologic entity common to many disease processes, including age-related macular degeneration (AMD), ocular histoplasmosis syndrome, degenerative myopia, multifocal choroiditis, and angioid streaks (Cohen et al., 1996; Ryan, 1976). The most common of these, AMD, is a leading cause of vision loss that affects more than 11 million people in the United States (Allikmets et al., 1997; Ferris et al., 1984; Macular Photocoagulation Study Group, 1991). Dry AMD, the more common form of the disease, is characterized by drusen, pigmentary and atrophic macular changes, and a slow progressive loss of central vision. Wet, or exudative, AMD is less common, but it accounts for 80% of severe vision loss caused by AMD. The wet form is characterized by subretinal hemorrhage, fluid, and fibrosis secondary to CNV and is associated with a more rapid and pronounced loss of central vision.

A variety of pharmacologic, gene-based, surgical, and radiation treatments are being developed to target CNV (Hollick

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et al., 1996; Ishida et al., 1999; Murata et al., 2000; Saishin et al., 2003; Yasukawa et al., 2002). Suitable animal models are needed to evaluate the efficacy of such treatments early in the development process. The rabbit offers a simple, inexpensive, large animal model for many ocular diseases. A large eye model is of particular importance for evaluating treatments based on instrumentation or devices.

Laser-induced damage to Bruch's membrane has been used to create CNV in primates, mice, and rats (Campochiaro, 2000; Frank et al., 1989; Ishibashi et al., 1987; Ishida et al., 1999; Zacks et al., 2002), but this technique has been unsuccessful in rabbits Angiogenic molecules, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), have been used successfully to induce subretinal CNV in rodents and primates (Cui et al., 2000; Wang et al., 2003); but the reproducibility and efficiency of these methods in rabbits have not been as promising (elDirini et al., 1991;Tamai et al., 2002).

Vascular endothelial growth factor plays a central role in new blood vessel formation (Ozaki et al., 1998; Saishin et al., 2003). It is a major stimulator of CNV in patients (Frank et al., 1996; Kvanta et al., 1996; Lopez et al., 1996), as well as in some animal models (Kwak et al., 2000; Miller et al., 1994; Okamoto et al., 1997). Intravitreal sustained release of VEGF, however, produces only retinal, not choroidal, neovascularization in rabbit eyes (Okamoto et al., 1997).

Matrigel is a solubilized basement membrane matrix extracted from the Engelbreth–Holm–Swarm (EHS) mouse sarcoma, a tumor rich in extracellular matrix proteins. Matrigel was developed to study endothelial cell differentiation in vitro and angiogenic processes in vivo (Kleinman et al., 1987; Passaniti et al., 1992). Its major matrix components are laminin, collagen 1, and heparin sulfate proteoglycan, along with growth factors such as bFGF, IGF, and EGF (Passaniti et al., 1992). Matrigel is a liquid at 4 °C; but it forms a solid gel at 37 °C, effectively trapping the growth factors and permitting their slow release. This slow-release characteristic of Matrigel makes it attractive as a means of simulating the natural angiogenic cascade response.

In the present study, we used Matrigel, both alone and in combination with VEGF, to stimulate subretinal CNV in the rabbit. This novel method provides a highly reproducible, reliable, and sustainable rabbit model of experimental CNV that will be of great usefulness in evaluating the effectiveness of new anti-angiogenic therapies when a larger animal eye is required.

2. Materials and methods

2.1. Preparation of subretinal delivery compounds

Matrigel basement membrane matrix (BD Biosciences, Bedford, MA) was thawed at 4 °C overnight. Human VEGF (Sigma, St. Louis, MO) was reconstituted in 1% bovine serum albumin in sterile phosphate-buffered saline (PBS) at 0.5 μg/μl in stock solution. Two VEGF/Matrigel suspensions were prepared: 500 ng of VEGF was suspended in 10 μl of Matrigel; 750 ng VEGF was suspended in 20 μl of Matrigel.

2.2. Experimental animal model

Twenty-two Dutch-belted pigmented rabbits, each weighing 5–7 lbs (2.3–3.1 kg), were used. The 19 rabbits that received Matrigel injection (with or without VEGF) were divided into four groups: Group 1 ($n = 8$): 20 μl Matrigel with 750 ng VEGF; Group 2 ($n = 3$): 10 μl Matrigel with 500 ng VEGF; Group 3 ($n = 5$): 20 μl Matrigel alone; Group 4 ($n = 3$): 10 μl Matrigel alone. The remaining three rabbits were injected with 20 μl PBS. The 22 rabbits were sequentially killed at weeks 1 ($n = 2$); 2 ($n = 5$), 4 ($n = 8$), and 9 ($n = 7$) from different groups (Table 1), and their eyes were subjected to histologic analysis.

All animal procedures used for this study adhered to the provisions of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the University of Southern California Institutional Animal Care and Use Committee (IACUC). The animals were anesthetized with an intramuscular injection of 35 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride. Their pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine hydrochloride eye drops. The ocular surface was additionally anesthetized with topical instillation of 0.5% proparacaine hydrochloride eye drops.

Under an operating microscope, a 3-mm paralimbal conjunctival incision was made in the supranasal quadrant in the right eye, and a sclerotomy was made 2 mm posterior to the corneal limbus. A 30-gauge needle attached to a Hamilton syringe (Hamilton Co., Reno, NV) was inserted through the sclerotomy site into the vitreous cavity. One of the two Matrigel VEGF suspensions or Matrigel alone was injected into the subretinal space about 1–1.5 optic disc diameters inferonasal to the disc. The injection resulted in a localized subretinal bleb/neurosensory retinal detachment. In the control animals, 20 μl of PBS was injected into the subretinal space at a similar location. Topical antibiotic was applied, and 10 mg gentamycin and 0.2 mg dexamethasone were injected subconjunctivally at the conclusion of the procedure.

2.3. Follow-up examination

Follow-up examination procedures included indirect ophthalmoscopy, stereoscopic fundus photography, fluorescein angiography (FA), and optical coherence tomography (OCT, Carl Zeiss Ophthalmic Systems, Dublin, CA). Prior to examination, the animals were anesthetized with an intramuscular injection of 35 mg/kg ketamine hydrochloride and 5 mg/kg xylazine hydrochloride, and their pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine hydrochloride eye drops. Indirect ophthalmoscopy was carried out on all animals at postoperative day 1 and weeks 1, 2, 4, and 9. Fundus photography, FA, and OCT (Zeiss OCT3) were performed on all animals at weeks 1, 2, 4, and 9, focusing on the injection site area. The greatest linear dimension (GLD) and disc areas (DA) (expressed as the smallest Macular Photocoagulation Study [MPS] DA that could completely encompass the lesion) of subretinal leakage were determined using measurement

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