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Oxygen-induced retinopathy in mice with retinal photoreceptor cell degeneration

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ABSTRACT

Aims: It is reported that retinal neovascularization seems to rarely co-exist with retinitis pigmentosa in patients and in some mouse models; however, it is not widely acknowledged as a universal phenomenon in all strains of all animal species. We aimed to further explore this phenomenon with an oxygen-induced retinopathy model in mice with retinal photoreceptor cell degeneration.

Main methods: Oxygen-induced retinopathy of colored and albino mice with rapid retinal degeneration were compared to homologous wild-type mice. The retinas were analyzed using high-molecular-weight FITC-dextran stained flat-mount preparation, hematoxylin and eosin (H&E) stained cross-sections, an immunohistochemical test for vascular endothelial growth factor (VEGF) distribution and Western blotting for VEGF expression after exposure to hyperoxia between postnatal days 17 (P17) and 21.

Key findings: Leakage and areas of non-perfusion of the retinal blood vessels were alleviated in the retinal degeneration mice. The number of preretinal vascular endothelial cell nuclei in the retinal degeneration mice was smaller than that in the homologous wild-type mice after exposure to hyperoxia ($P < 0.01$). The degree of oxygen-induced retinopathy was positively correlated with the VEGF expression level. However, the VEGF expression level was lower in the retinal degeneration mice.

Significance: Proliferative retinopathy occurred in mice with rapid retinal degeneration, but retinal photoreceptor cell degeneration could partially restrain the retinal neovascularization in this rapid retinal degeneration mouse model.

Introduction

Angiogenic diseases such as retinopathy of prematurity, proliferative diabetic retinopathy and neovascular age-related macular degeneration are common causes of blindness. Previous reports have stated that retinal degeneration might inhibit the development of retinal angiogenesis and neovascularization (Lahdenranta et al., 2001; Arden, 2001). It has been proposed that rod photoreceptor cells consume more oxygen under dark adaptation, and that the increased oxygen consumption leads to retinal hypoxia (Arden et al., 2005). Retinal hypoxia is an important state that increases the expression levels of hypoxia inducible factor- (HIF-) regulated growth factors, such as vascular endothelial growth factor (VEGF) and erythropoietin (EPO), which ultimately leads to retinal neovascularization. Conversely, when retinal photoreceptor cells degenerate and oxygen consumption decreases, a hyperoxic state appears in the retinal microenvironment. Hyperoxia leads to down-regulation of the expression levels of oxygen-regulated growth factors such as VEGF and EPO, which might give rise to retinal vascular suppression and obliteration. Previous reports proposed that vascular atrophy could occur in a rapid retinal degeneration mouse model (Blanks and Johnson, 1986). It was hypothesized that there was an anti-angiogenic state in mice and humans with retinal photoreceptor cell degeneration (Lahdenranta et al., 2001). Furthermore, it has been reported that blood vessel development was restrained in the rapid retinal degeneration mouse model and less restrained in slow retinal degeneration rat and mouse models (Pennesi et al., 2008). Another report found that the loss of the outer retina alleviated the severity of diabetic retinopathy in a $Rho^{-/-}$ mouse model (de Gooyer et al., 2006). Pennesi et al. investigated the relationship between photoreceptor degeneration and retinal vascular development in different retinal degeneration animal models and concluded that the vascular profiles began to fade when less than approximately 30–33% of the photoreceptors were retained. In two mouse lines with early-onset and rapid photoreceptor loss, S334ter-7 and S334ter-3, in which approximately 90% and 65%, respectively, of the photoreceptors were lost by postnatal day (P) 15, the capillary plexus failed to form (S334ter-7) and the number of capillary profiles was less than 7% compared with controls (S334ter-3). The effect of photoreceptor absence was permanent, and there was no late

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vascularization (Pennesi et al., 2008). However, there are reports that found that retinal neovascularization occurred in patients with retinitis pigmentosa (Kadayifcilar et al., 2000; Hayakawa et al., 1993) and in a retinal degeneration mouse model (Nishikawa and LaVail, 1998). On the basis of the above reports, it seems that the relationship between photoreceptor cell degeneration and retinal neovascularization is controversial. In the present study, we used albino and colored inbred mouse strains with retinal photoreceptor cell degeneration to investigate the relationship between retinal neovascularization and retinal photoreceptor cell degeneration. Our results suggest that the loss of photoreceptor cells cannot completely obstruct retinal neovascularization in a mouse model of rapid retinal degeneration.

Materials and methods

Animals

This study adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Mouse experiments were approved by the Animal Care and Use Committees of Fourth Military Medical University. The retinal degeneration mouse model (Kunming mice) used in this study had rapid retinal degeneration caused by an autosomal recessive disease of photoreceptor cell degeneration, and these mice have a spontaneous mutation of the *pde6b* gene that was discovered in 2003 (Yan, 2008; Yan et al., 2008). Subsequently, the mutant mouse was mated to generate an inbred strain. We temporarily named it "retinal degeneration fast mouse" (Pde6b^{rdf}/Pde6b^{rdf}, rdf/KM). To identify the causative gene for further research, we established a congenic inbred strain with the C57BL/6 J background and temporarily named it rdf/C57. The mutant mouse model is similar to the well-known rd1 mouse model with an autosomal recessive disease of photoreceptor cell degeneration. The progression of disease and the degree of photoreceptor cell degeneration in Pde6b^{rdf} mice are similar to those in rd1 mice (Fig. 1A). The mutant mice used in this study were from the F14–F16 generations of Pde6b^{rdf}/Pde6b^{rdf} mice in the Barrier Animal Laboratory of the Department of Clinical Aerospace Medicine. Wild-type C57BL/6 J mice and Kunming mice provided by the Animal Center of the Fourth Military Medical University were used as controls. A blank control was housed in room air for each strain. Twenty pups from each strain (eighty pups in total) were used in this experiment. Pups of both sexes were utilized in the following experiments.

Analysis of photoreceptor cell development in Pde6b^{rdf}/Pde6b^{rdf} mice

To observe the morphological changes in the retinas of Pde6b^{rdf}/Pde6b^{rdf} mice during development, six rdf/C57 and six rdf/KM mice pups were sacrificed at each of the following time points: P7, P10, P12, P14, P15, P17 and P21. Homologous wild-type mice of each strain were also sacrificed at matching time points. After paraformaldehyde

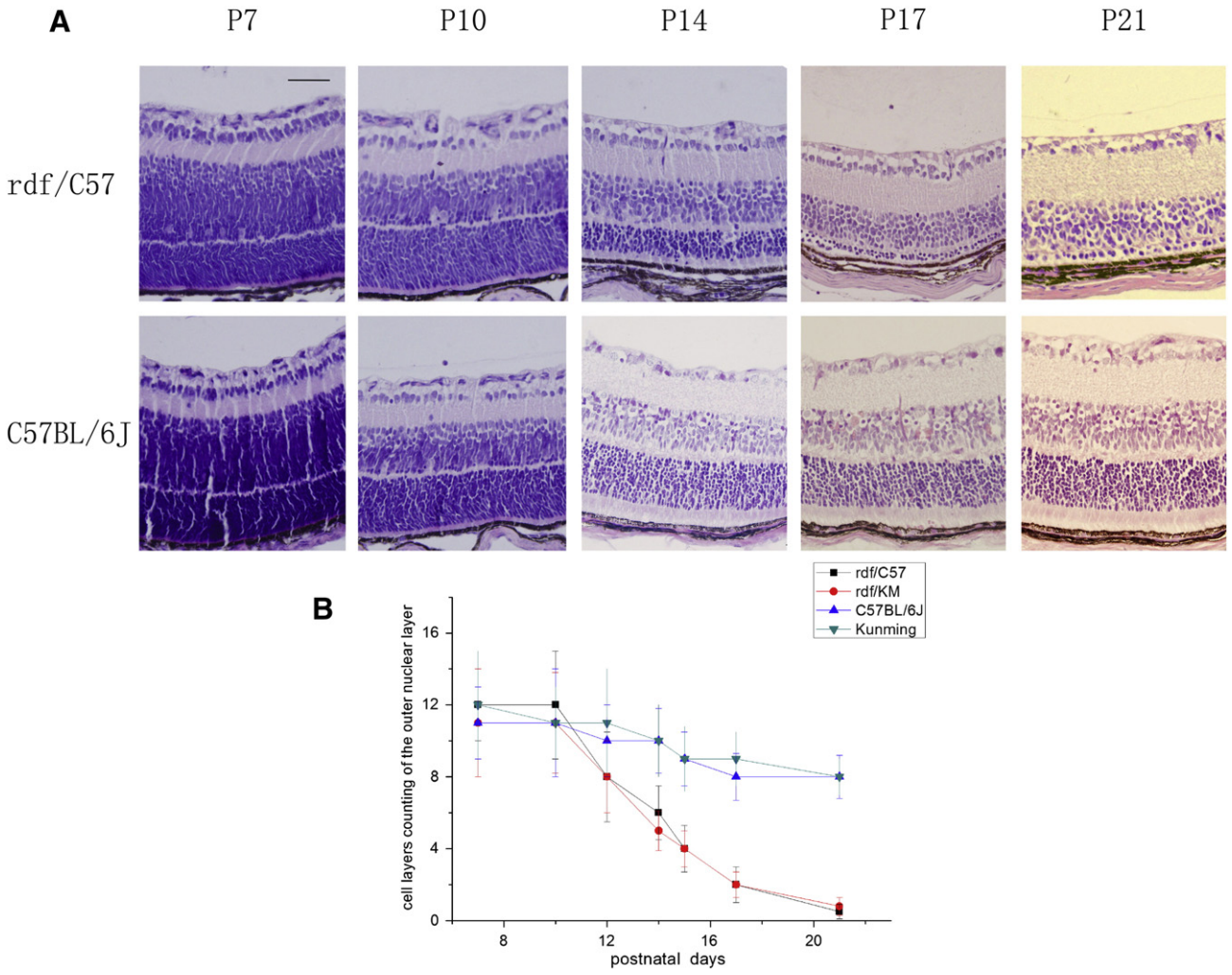

Fig. 1. (A) The development of retinal ONL in rdf/C57 mice and C57BL/6 J mice from P7 to P21. Scale bar = 50 μm. (B) Retinal cell layers in the ONL of the retina during the development of Pde6b^{rdf}/Pde6b^{rdf} mice and the homologous wild-type mice.