

# Inhibition of retinal angiogenesis by PEDF

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## Abstract

• **AIM:** To investigate the effect of PEDF on retinal neovascularization in mice.

• **METHODS:** 40 7-day-old C57BL/6J mice was exposed to 750mL/L oxygen for 5 days and then to normal situation to produce the murine model of oxygen-induced retinopathy (OIR). One eye of the mouse was regarded as experimental one and the other served as control. Eyes in experimental group received intravitreal injection of PEDF and eyes in control group received intravitreal injection of PBS at postnatal day 12. All mice were executed at postnatal day 17. The changes of retinal vessels of mice were observed by ADPase histochemical technique. The inhibitory effect of PEDF on retinal neovascularization was evaluated by counting the endotheliocyte nuclei of new vessels which extended from retina to vitreous in the tissue slice of HE staining.

• **RESULTS:** Neovascularization was reduced, retinal blood vessels distributed regularly and non-perfusion areas were not found in eyes of experimental group compared with control group. The number of endotheliocyte nuclei of new vessels extending from retina to vitreous was significantly less in the eyes of experimental group ( $10.18 \pm 1.74$ ) than that in control group ( $38.89 \pm 2.98$ ) ( $P < 0.01$ ). Retinal toxicity and inflammatory reactions were not found in tissue slice.

• **CONCLUSION:** PEDF inhibits retinal angiogenesis in OIR and the feasibility should be determined for use of PEDF in ocular angiogenesis treatment.

• **KEYWORDS:** PEDF; retinal neovascularization

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## INTRODUCTION

Neovascular diseases of the retina collectively constitute the leading cause of blindness in developed countries<sup>[1]</sup>. At present, retinal laser photocoagulation appears to be the most effective treatment for retinal neovascularization. However, this procedure can destroy postmitotic retinal neurons and permanently affect visual function. Pharmacologic agents that inhibit angiogenesis without destroying retinal tissue could lead to new treatments for this constellation of diseases. Because angiogenesis is a multistep process regulated by an array of growth factors and extracellular matrix molecules<sup>[2-5]</sup>, there are potentially many ways to interfere with its progression. Recently, a number of investigators have partially inhibited retinal and iris angiogenesis *in vivo* using monoclonal antibodies, receptor-binding proteins, and antisense oligonucleotides to vascular endothelial growth factor (VEGF), an endothelium-specific growth factor. Pigment epithelium-derived factor (PEDF) is potentially both a promising endogenous inhibitor of angiogenesis and a neuroprotective protein. Many studies have demonstrated that it is a potent antiangiogenic agent that inhibits the migration of endothelial cells *in vitro* and promotes the apoptosis of vascular endothelial cells and has been a more potent antiangiogenic agent than angiostatin, thrombospondin-1, or endostatin in assays. In this study, we examined the effect of PEDF on animal models of retinal neovascularization.

## MATERIALS AND METHODS

### Mouse Model of Oxygen-induced Ischemic Retinopathy

Ischemic retinopathy was produced in C57BL/6J mice by a method described by Smith *et al*<sup>[6]</sup>. Forty seven-day-old (postnatal day 7) mice and their mothers were placed in an airtight incubator and exposed to an atmosphere of  $(750 \pm 50)$  mL/L oxygen for 5 days. The incubator temperature was maintained at  $(23 \pm 2)^{\circ}\text{C}$ . Then they were returned to room air at postnatal day 12. One eye was treated as experimental one and the other served as control. At the 12th day, eyes in experimental group received intravitreal injections of PEDF  $1\mu\text{L}$  ( $2.5\text{mg/L}$ ) and eyes in control group received intravitreal injections of PBS  $1\mu\text{L}$ .

**Observation of Retinal Neovascularization** At the 17th day, 10 mice were anesthetized with an intramuscular injection

tion of ketamine (80mg/kg) and xylazine (15mg/kg). To evaluate vessel morphology, all eyes were removed and fixed with 40g/L paraformaldehyde in phosphate-buffered saline overnight. The cornea, lens, and vitreous were surgically removed and retinas were dissected. Retinas were processed for magnesium-activated adenosine diphosphate (ADPase) staining as previously described by Luty and McLeod<sup>[7]</sup>. ADPase-stained retinas were flatmounted on microscope slides with a gelatin-coated cover slip. The vasculature was then examined under microscope.

**Quantification of Retinal Neovascularization** On the 17<sup>th</sup> day, 30 mice were sacrificed and the eyes were enucleated, immersed in 40g/L paraformaldehyde in PBS for at least 24 hours, and embedded in paraffin. Serial 6 $\mu$ m sections from all eyes were cut sagittally parallel to the optic nerve and stained with hematoxylin and eosin according to a standard protocol. The extent of neovascularization was determined by counting neovascular cell nuclei extending through the internal limiting membrane into the vitreous. All counting was done based on a masked protocol. For each eye, 10 intact sections of equal length, each 30 $\mu$ m apart, were evaluated. The mean number of neovascular nuclei per section per eye was then determined. Ten percent of the eyes exhibited retinal detachment or endophthalmitis and were excluded from the evaluation.

**Statistical Analysis** All values were expressed as mean  $\pm$  SD. All analyses were performed with appropriate software SPSS. The extent of neovascularization in PEDF-treated eyes was compared with that in control eyes using paired student's *t*-test. A value of  $P < 0.05$  was considered statistically significant.

## RESULTS

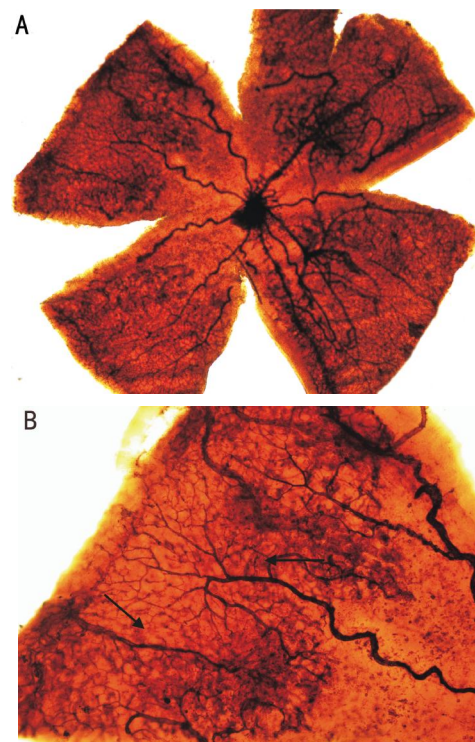
Forty murine models of oxygen-induced retinopathy were established successfully.

### ADPase Histochemical Technique in Retinal Flatmounts

The pattern of vascular development and neovascularization were seen readily in retinal flat-mounts by ADPase histochemical technique. The retina of mice in experimental group at the 17<sup>th</sup> day had both superficial and deep vascular layers that extended from the optic nerve to the periphery. The vessels formed a fine radial branching pattern in the superficial retinal layer and a polygonal reticular pattern in the deep retinal layer, non-perfusion area and neovascularization were rarely seen in all eyes of experimental group (Figure 1). The retinal vascular pattern in the mice of control group was characterized by the neovascular lines, avascular area, microaneurysm and hemorrhage that represent a typical pattern of pathological retinal neovascularization (Figure 2).



**Figure 1 Flat-mounted, ADPase-stained retina in experimental group** A: The vessels formed a fine radial branching pattern in the superficial retinal layer and a polygonal reticular pattern in the deep retinal layer; no avascular area and little neovascularization were seen ( $\times 10$ ) B: peripheral retina developed well ( $\times 20$ )



**Figure 2 Flat-mounted, ADPase-stained retina in control group** A: The figure shows a typical pattern of pathological retinal neovascularization, including dilated and tortuous vessels and avascular area around the optic disc ( $\times 10$ ) B: The arrow indicates pre-retinal neovascularization which occurred near avascular area ( $\times 20$ )

Tissue Slice of HE Staining In the mice of experimental group, there was an average of  $10.18 \pm 1.74$  nuclei extending from retina to vitreous in the tissue slice of HE staining, significantly less than that ( $38.89 \pm 2.98$ ) in the mice of control group ( $P < 0.01$ ).

### DISCUSSION

PEDF, a protein with substantial homology to serine protease inhibitors (serpins), was first identified as a component of conditioned media of cultured fetal RPE cells that causes neurite outgrowth of Y79 retinoblastoma cells [8]. Several studies have suggested that PEDF has neuroprotective activity [9-11]. The RPE cells provide trophic support for photoreceptors and loss of RPE cells results in photoreceptor degeneration. PEDF might contribute to this trophic activity because it is produced by RPE cells, and in an *in vitro* model of photoreceptor degeneration in which the RPE is removed from *Xenopus* eyecups while PEDF protected photoreceptors from degeneration and loss of opsin immunoreactivity [12]. PEDF may also have other functions. The cDNA for PEDF was independently isolated from fetal lung cells in a screening for genes differentially expressed in nonproliferating, early population doubling cells compared to senescent cells. PEDF levels are high in growth-arrested cells and low in active ones as negative growth regulator [13]. Most important of all, PEDF promotes the apoptosis of vascular endothelial cells and inhibits vascular endothelial cell proliferation and migration [14]. It inhibits corneal neovascularization in a corneal pocket model, suggesting that it could be an endogenous inhibitor of angiogenesis [15]. In this study, we have demonstrated that PEDF can inhibit retinal angiogenesis in oxygen-induced retinopathy. Retinal neovascularization occurs in the setting of ischemic retinopathy, including diabetic retinopathy, central and branch retinal vein occlusion, and retinopathy of prematurity. Diabetic retinopathy is the most common cause of severe visual loss in young patients in developed countries. Scatter photocoagulation can often prevent severe visual loss from retinal neovascularization when given in a timely fashion, but is baffled by vitreous hemorrhage or other problems that impair visualization of the reti-

na, and in patients with very severe ischemia, sometimes scatter photocoagulation is not enough. PEDF may provide a useful adjunct to scatter photocoagulation in high-risk situations.

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