Chapter 15

OXYGEN-INDEPENDENT ANGIOGENIC STIMULI

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Abstract: Although much research has focused on the role of hypoxia and hyperoxia in preretinal neovascularization, there is growing evidence that other factors play a role. Carbon dioxide, acidosis, alkalosis, systemic infection, systemic growth retardation, and perturbations in the thyroxine and insulin-like growth factor (IGF-1) hormone axes all appear to be important risk factors in the pathogenesis of retinopathy of prematurity (ROP) and inducers of preretinal neovascularization in the immature retinae. Further advances in the prevention of ROP may require interventions directed at these oxygen-independent angiogenic stimuli.

1. INTRODUCTION

Retinopathy of prematurity (ROP) is a blinding disease of premature infants that, in its advanced stages, is characterized by preretinal neovascularization. Although excess inspired oxygen was identified as the primary risk factor for development of ROP almost 50 years ago,1,2 reduction of supplemental oxygen exposure for premature infants has failed to eliminate severe ROP.3,4 Multivariate analyses of retrospective clinical datasets have raised many alternative candidate risk factors in the pathogenesis of ROP, but such retrospective studies are limited by lack of independence of potential risk factors and incomplete data acquisition.

Animal models of ROP provide an opportunity to study individual candidate risk factors, while allowing control of other potential confounders. The rat model for ROP has been described in previous chapters of this text. To briefly restate the critical features: the retinal vasculature of the neonatal
rat is incompletely developed at birth, with a large avascular peripheral retina analogous to the premature human infant. Studies using this model make the assumption that exposing the neonatal rat retina to stimuli (e.g. hyperoxia) during the first few days of life is analogous to exposing premature human retina to those stimuli. Neovascularization in the rat model primarily develops at the junction of the vascular and avascular retinas, in the same way that stage 3 ROP develops in the retinas of human premature infants.

Several laboratories have studied the role of fluctuating hyperoxia and hypoxia on the development of preretinal neovascularization in the rat model, a condition termed “oxygen-induced retinopathy” (OIR). In most OIR rat models, newborn pups are exposed to periods of hyperoxia, alternating with periods of absolute or relative hypoxia, for a total of 7 to 14 days, and then retinas are evaluated after a further period of room air recovery ranging from 0 to 6 or more days. In our laboratory, the period of oxygen exposure is 7 days, with 5 days of recovery, and analysis at day 13 using primarily ADPase staining methods and masked grading. We have primarily used an “expanded litter” design, where rats are raised in foster litters of 25 by one mother. Such expanded litters induce growth retardation, which we have found to be associated with increased incidence and severity of neovascularization. We believe that standardizing this growth retardation is important, since animals raised in different sized litters have different rates of vascular development.

OIR in rats and mice appears to be mediated primarily by vascular endothelial growth factor (VEGF), analogous to ROP in premature infants. Nevertheless, other non-hypoxic, non-hyperoxic stimuli also appear to induce neovascularization in the neonatal rat, and many of these stimuli have clinical relevance to ROP in premature infants.

In any discussion of “oxygen-independent” stimuli, a caveat is needed. To date, there is no direct evidence that the stimuli we describe are mediated secondarily by hypoxia or hyperoxia. Nevertheless, it is entirely possible that some of these stimuli might be acting through local changes in the oxygen environment. With that caveat, we will describe a number of oxygen-independent factors that induce preretinal neovascularization in neonatal rats, providing additional animal models of ROP.

2. **CARBON DIOXIDE**

Premature infants who never experience hyperoxia, for example those with cyanotic congenital heart disease, may develop ROP. For those specific infants, and for premature infants in general, raised arterial carbon dioxide