Oxygen-induced retinopathy in the rat: Possible contribution of peroxidation reactions

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Abstract. Albino rats were maintained in 60% atmospheric oxygen from birth through 14 days of age. Age-matched controls were simultaneously raised in room air. Some rats were perfused with India ink before sacrifice and retinal dissection in order to study the effect of oxygen-rearing on the retinal vasculature. By this method it was found that oxygen-reared animals sustained a 36% loss of retinal blood vessels. Other animals' retinas were removed immediately after sacrifice and examined for evidence of lipid peroxidation by one of three means: 1) a determination of the presence of products of lipid peroxidation, 2) a measure of the loss of polyunsaturated fatty acids, and 3) a determination of retinal vitamin E level. Each of these determinations indicated that peroxidation reactions had occurred in the retinas of oxygen-reared rats. Retinal vitamin E was supplemented in the young rats through the diet of the mothers. This treatment resulted in a two-fold increase of retinal vitamin E over levels in pups of mothers fed rat chow. Oxygen-reared vitamin E-supplemented rats sustained significantly less obliteration of blood vessels than non-supplemented oxygen-reared animals.

Introduction

Over the last two years, we have been recharacterizing the rat as an animal model for retinopathy of prematurity. Under the proper conditions, the retinal integrity of hyperoxic rats is compromised in three ways: a temporary absence of retinal blood vessels [1, 11, 12], a permanent ERG b-wave deficit [12], and an expression of GFAP by Müller cells [12]. Each of these effects indicates a disturbance of the normal development of the inner retina, and this should not surprise us since the direct effect of oxygen on the immature retina – an interruption of the supply of blood-borne nutrients – occurs there. The purpose of the experiments described herein was to begin to test the possible role of peroxidation reactions as the cause of these disturbances.

The retina is particularly susceptible to oxygen-mediated damage for several reasons: 1) its cells have a very rapid rate of oxygen consumption,
even under normoxic conditions [13]; 2) it has the highest levels of polyunsaturated fatty acids of any known tissue, and these molecules are preferred substrates for peroxidation [6]; and 3) it processes light, a known initiator of oxygen radical formation [7]. Under elevated levels of oxygen, the first aspect, that of oxygen flux, is likely enhanced. The importance of oxygen-mediated damage to the retina is also inferred from its elaborate defense system. The retina is able to combat peroxidation by enzymatic detoxification of reactive oxygen molecules or by termination of radical chain propagation by vitamin E. The rationale for using vitamin E as treatment for retinopathy of prematurity stems from the idea that peroxidative damage to cells is a causal factor and that, by acting as a scavenger of the reactive molecules that lead to peroxidation, vitamin E may prevent this damage. Oxidative damage to cells is often attributed specifically to peroxidation of the polyunsaturated fatty acids in the lipid of membranes, which causes loss of fluidity and breakdown of membrane secretory functions that control transmembrane ionic gradients [3]. Since vitamin E is an integral part of the lipid bilayer, it can react with the radicals there by transferring a hydrogen atom to them. Vitamin E then becomes a radical itself, but, because of its relative stability, it is unable to continue the chain propagation of lipid peroxidation.

Direct evidence from animal models that free radicals are involved in retinal oxygen toxicity has come from several studies. Bougle and coworkers [5] demonstrated a reduction of retinal superoxide dismutase (SOD) activity in newborn kittens exposed to 72 hrs of 80% oxygen. This suggested a reduced ability of the kitten retina to defend itself against attack by oxygen radicals. Previous supplementation with vitamin E protected against loss of retinal SOD. Taki [14] has described increased levels of lipid peroxides in the retina and blood of kittens exposed to 48 hrs of 70% oxygen from day 3 of life. He has suggested a free radical reaction with the lipids of cell membranes as the source of these peroxidases. If a subcutaneous injection of tocopherol acetate was made during and after exposure, the levels were reduced. Yagi and coworkers [15] have reported elevated lipoperoxide levels in both the blood and the retina of chick embryos on exposure to 95% oxygen for 12 or 24 hours. Finally, Hiramatsu and colleagues [9] have also measured increases in lipoperoxides in rabbit retinas following 12 hours at 90–95% oxygen.

We have chosen to test the presence of peroxidation reactions in oxygen-reared rats by a combination of three separate means. These are: 1) a determination of the presence of products of lipid peroxidation in the retina, 2) a measure of the loss of retinal substrate for peroxidation reactions, and 3) a determination of the retinal vitamin E level. Having found that retinal