Aberrant Retinal Angiogenesis, Structure, and Synaptic Morphology in an in vivo Mouse Model of ROP

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Objective: Retinopathy of prematurity (ROP) is a common condition of premature infants. Vascular endothelial growth factor (VEGF) is a major angiogenic factor in both physiologic and pathologic retinal angiogenesis including ROP. However, cellular changes that take place in ROP are not fully understood. The aim of this study is to understand changes in retinal morphology, vasculature, and structure in mice with oxygen-induced retinopathy (OIR), a model for ROP.

Methods: Twenty-two mice underwent hyperoxia exposure at 77% to induce OIR, while twenty-three age-matched control mice were raised in room air (RA). After anesthesia, pupils were dilated and fluorescein was injected intraperitoneally. After in vivo imaging at P19, P24, P32, and P47, eyes were isolated and fixed in Bouin’s fixative for morphologic analysis by H&E staining, and paraformaldehyde for immunohistochemistry. For immunohistochemistry, markers for photoreceptor presynapses (PSD95), bipolar cells (PKCalpha), microglia (Iba1), and vascularization (VEGF) were used to investigate synaptic abnormalities, inflammation, and vascularization. To detect apoptosis, ApopTag Fluorescein In Situ Apoptosis Detection Kit (Millipore) was used.

Results: OIR mice had a vascular-avascular retinal phenotype, with more dilated veins and more tortuous arteries than RA mice in early ages. The OIR Retinas showed disorganized synaptic interactions between photoreceptor cells and bipolar cells, with significant thinning in the ganglion cell, inner nuclear, outer plexiform, and outer plexiform layers. Retinal cross-sections showed altered structure in OIR mice, with dilated blood vessels and avascularization in the inner retina. There was significantly increased microglia activity in ganglion cell, inner nuclear, and outer plexiform layers. There was increased apoptosis in OIR mice, which decreased over time, but persisted until P47. There was increased VEGF expression in bipolar cells. TUNEL staining showed increased apoptosis in OIR mice than RA mice at P19 (P<0.001), P24 (P<0.001), P32 (P<0.001), and P47 (P<0.001). Apoptosis decreased over time with developmental maturity of the OIR mice, but persisted at P47.

Conclusion: Our results demonstrate patchy vascular-avascular phenotype, defective synaptic interactions and persistent apoptosis in the OIR retina, which affected retinal vasculization, and structure in the long term. Our study aids the understanding of cellular and molecular complexities of the retina following oxidative stress, and could lead to potential new therapeutic targets.