An Improved Mouse OIR Model that Avoids any Need for Surrogate Dams

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Abstract

Objectives
To investigate the therapeutic potential of recombinant Norrin in rescuing the retinal vasculature in a mouse model of oxygen-induced retinopathy (OIR), modified to prevent loss of dams from pulmonary inflammation.
Methods
Mouse pups were subjected to the OIR protocol (75% oxygen from P7-P12). At P13, mice received an intravitreal injection of Norrin protein or a vehicle control. A key modification involved cycling dams to room air for 1.25 hr each day to prevent development of pulmonary inflammation in the adult mice. At P17, retinas were harvested, flat-mounted, and stained with Alexa Fluor 594-conjugated isolectin B4 to visualize the vasculature for quantification via confocal microscopy.
Results
The intravitreal injection procedure was executed successfully. Post-procedural examination confirmed that the vitreous humor was injected with little to no hemorrhaging observed at the injection site. Further ocular testing using fluorescein angiography, optical coherence tomography (OCT), revealed no damage to non-retinal ocular structures due to the injection. All dams were free of any signs of pulmonary inflammation.
Conclusion
There was no need to use surrogate dams with the modified protocol and Norrin reduced avascular area compared to vehicle as in our previously published studies. Confocal imaging also improved the detail of the retinal microvasculature in flat mounted retinas compared to regular epifluorescence microscopy.

introduction

Norrin is a secreted signaling protein and a potent ligand for the Wnt/β-catenin pathway, which is essential for the proper development and stabilization of the retinal vasculature. To study diseases of retinal angiogenesis, such as Retinopathy of Prematurity (ROP), the murine model of Oxygen-Induced Retinopathy (OIR) is widely utilized. This model effectively recapitulates neovascular phase of clinical pathology in retinovascular diseases such as fetal exudative vitreoretinopathy (FEVR), Norrie's disease, and ROP. Central vaso-obliteration is generated with hyperoxia, followed by hypoxia-driven pathological neovascularization upon return to room air. Given Norrin's fundamental role in physiological vessel growth, evaluating its therapeutic potential to promote healthy revascularization in the OIR model is a critical area of research. One undesired nature of the model is the fact that the adult dams often develop oxygen induced pulmonary inflammation due to a shift in lung microbial flora to proinflammatory streptococci. Daily room air breaks for dams were introduced to prevent lung inflammation.

Methods

All experiments were conducted with approval of the OU IACUC. The Oxygen-Induced Retinopathy (OIR) model was established by placing postnatal day 7 (P7) litters in a 75% oxygen chamber for five days (P7-P12). Dams (not pups) were cycled to room air for 1.25 hours (21% oxygen) each day. Anesthetized mice received a single intravitreal injection at P14 in the OD eye, where mice were injected with either recombinant human Norrin or vehicle while the fellow eye was an uninjected control. At P17 retinas were harvested for flat-mount analysis. The retinal vasculature was labeled with Isolectin-B4-Alexa594, mounted flat and then imaged by digital stitching with a Zeiss confocal microscope. Areas of total retinal area and avascular areas were quantified using the GNU Image Manipulation Program.

Methods

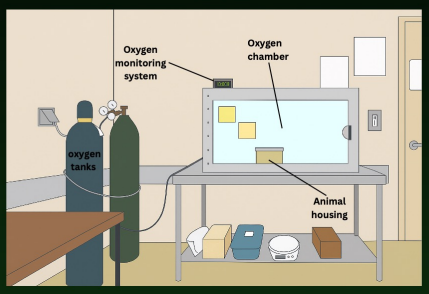

Figure 1. OIR mouse model illustration of a typical Hyperoxia chamber. hyperoxia chamber system used to induce retinopathy in neonatal mouse pups. Pressurized oxygen tanks supply oxygen to a sealed chamber where the mice are housed with their nursing mother. An oxygen monitoring system precisely controls the internal atmosphere, maintaining a constant 75% oxygen level throughout the 5-day hyperoxic exposure period (P7-P12). This controlled environment is essential for inducing the initial vaso-obliteration phase of the OIR model.

Figure 2. Confocal Imaging System and Analysis of OIR-Induced Retinal Pathology.
This illustrated the confocal microscope and its associated computer workstation used to acquire the are shown, illustrating the equipment used for retinal imaging.

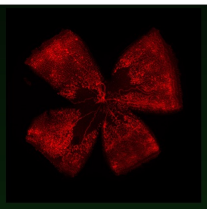

Figure 3. Example of a P17 mouse retina from an Oxygen-Induced Retinopathy (OIR) model, stained with isolectin B4 to visualize retinal endothelial cells of the vasculature. The central non-perfused region indicates the avascular area, which is the metric used to calculate the avascular fractional area.

Results

This improved mouse OIR model solves it's common problem of the loss of dams to oxygen induced pulmonary inflammation. The OIR model itself was still functional with the expected vasobliteration and neovascular regrowth phases. A test with Norrin treatment successfully reduced avascular area at P17 as seen previously using the classic protocol. Norrin reduced the avascular fraction by 20% compared to vehicle. This improved OIR model is now in use to test therapeutic proteins based on human Norrin.

Discussion & Conclusion

Our intravitreal injection protocol proved to be safe and well-tolerated in the OIR mouse model. The procedure caused minimal trauma, preserving the retinal structure. The modified model completely prevents the occurrence of pulmonary inflammation and does not require replacing dams with surrogates. The modified protocol is a reliable method for delivering therapeutic agents such as norrin protein in future preclinical studies of ischemic retinopathies.

Citations

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