Phosphate-buffered saline administered intravitreally at P12 blocks neovascularization in the mouse oxygen-induced retinopathy model

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Purpose

Intravitreally injected phosphate-buffered saline (PBS) is known to decrease neovascularization in the mouse oxygen-induced retinopathy (OIR) model[1,2], and it is commonly used as a vehicle control in preclinical studies evaluating therapeutic efficacy. This study aimed to evaluate the impact of intravitreal (IVT) PBS administration on neovascularization (NV) and avascular areas (AVA) at different timepoints after OIR induction. Aflibercept (Eylea®) was used as a reference control.

Methods

Male and female C57BL/6JRj mice were exposed to 75% oxygen (BioSpherix ProOx) from postnatal day 7 (P7) for a period of 5 days. On P12, the mice were returned to normoxic conditions and received IVT injections of either PBS or aflibercept in the right eye on P12, P13, or P14 (n=7-9 eyes/group). The left eye was left untreated as a contralateral control. At P17, retinal flat mounts were collected and stained with Isolectin B4. Microscopic images of the whole retina were then analyzed for neovascular (NV) and avascular (AVA) areas using a proprietary AI-based algorithm. The results were analyzed with Mann-Whitney test or unpaired t-test separately at each time point.

Results

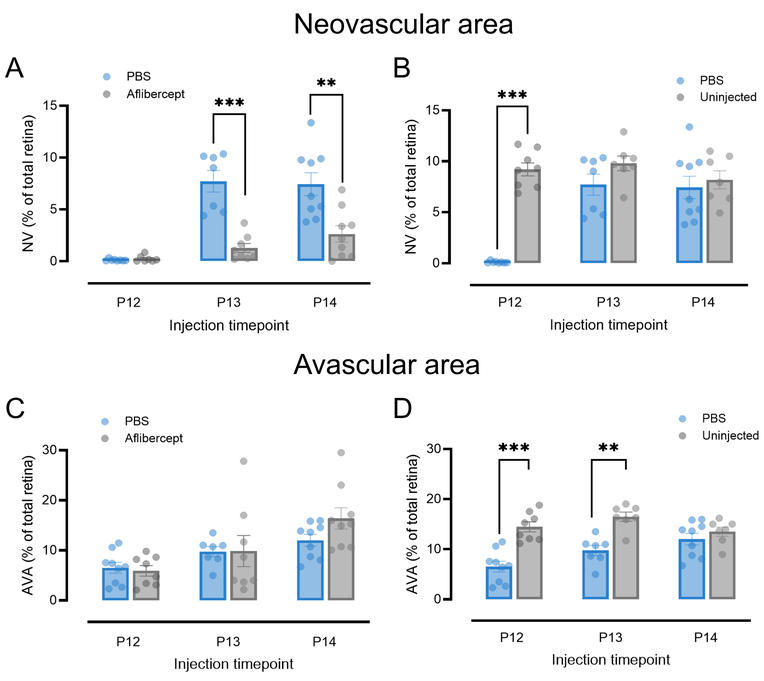

Figure 1. Neovascular (A-B) and avascular (C-D) data are presented as mean±SEM from 7-9 retinas per group. Data were analyzed by Mann-Whitney test or unpaired t-test comparing PBS to aflibercept or injected eyes to uninjected eyes at each timepoint separately (** P<0.01, *** P<0.001).

- The effect of PBS on NV and AVA areas was similar to aflibercept when administered at P12
- Aflibercept, injected at P13, reduced NV by 83% (P<0.001) and by 65 % injected at P14 (P<0.01) when compared to PBS, whereas AVA remained similar between groups
- When compared to uninjected eyes, PBS significantly reduced NV at P12, and the size of AVA area at P12 and P13 (P≤0.001). No significant group differences were evident at P14

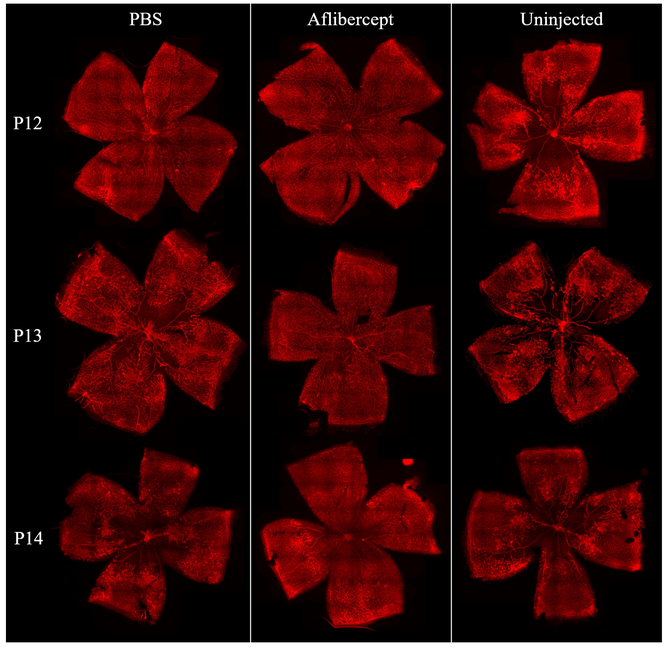

Figure 2. Representative images of Isolectin B4-stained retinal flat mounts at P17 from the PBS and aflibercept groups, injected at P12, P13, and P14, along with representative images from uninjected eye retinas.

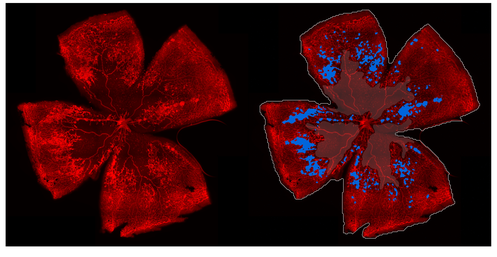

Figure 3. Representative image of an Isolectin B4 stained retina (uninjected) analyzed for NV and AVA area by a proprietary AI-based algorithm. Masks are marked on the right for NV area (blue) and AVA area (grey). The total area of the retina is outlined in white.

Conclusion

Our findings showed a significant anti-angiogenic effect of PBS when injected at P12. However, PBS administered at P13 or P14 did not block neovascularization, which was successfully inhibited by aflibercept. These results suggest that P12 is not an optimal timepoint for IVT treatment when PBS is used as a vehicle. Further studies are needed to explore if similar effect at P12 is seen with other vehicles or neutral antibody controls.

Disclosures
MV, BL, NJ, AT, XE, OV, AH, AAK, AMK, PP: none
GK: Experimentica Ltd. (I,S)

References
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