Aflibercept inhibits physiological revascularization and pathological neovascularization in the mouse and rat oxygen-induced retinopathy models

Maria Vähätupa,[1,2] Jenni J. Hakkarainen,[1] Simon Kaja,[1,3] Hannu Uusitalo,[2] Tero AH Järvinen,[2] Hannele Uusitalo-Järvinen,[2] and Giedrius Kalesnykas[1]

[1] Experimentica Ltd., Microkatu 1, P.O. Box 1199, FI-70211 Kuopio, Finland [2] Faculty of Medicine and Life Sciences, University of Tampere, Finland
[3] Departments of Ophthalmology and Molecular Pharmacology & Therapeutics, Stritch School of Medicine, Loyola University Chicago, Maywood, IL, USA

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Introduction

Mouse and rat oxygen-induced retinopathy (OIR) models are commonly used models to study ischemic retinopathies involving neovascularization, such as retinopathy of prematurity. Here the effect of aflibercept on retinal revascularization, neovascularization and microgliosis was studied in both the mouse and the rat model of OIR. Sham-injected or untreated eyes were used for treatment comparison.

Materials and Methods

Mouse and Rat OIR models

C57BL/6J mice (Janvier Labs, France) were exposed to 75% O_2 for five days from postnatal day 7 (P7) to P12.(1) Sprague Dawley rats (Charles River, Germany) were exposed to the alternating 50/10% oxygen protocol where O_2 levels are alternating between hyperoxia (50%) and hypoxia (10%) every 24h from P0 to P14(2), using an O_2 controller (BioSpherix ProOx Model 110, Biospherix Ltd., NY, USA).

Treatment administration

Intravitreal injections of Aflibercept (Eylea®, 20 µg/0.5 µl for mice and 40 µg/1.0 µl for rats) were performed at P14 under isoflurane anesthesia. Control groups were either injected with PBS, IgG control, punctured or left untreated. At the end of each study, mice were euthanized at P17 and rats at P20.

Quantification of vasculature and microglia

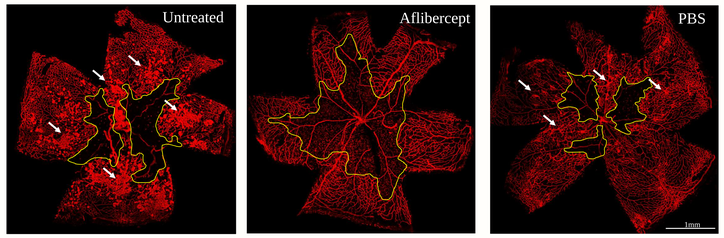
Retinas were flat mounted and stained with Isolectin GS-IB_4 (Invitrogen) for vasculature and Iba1 (Wako Chemicals) for microglia and imaged using confocal microscopy. Lasso and Magic Wand Tools in Adobe Photoshop CC software were used to select the avascular areas (AVAs) and preretinal neovascularization (NV). The total number of microglia was estimated using unbiased stereology. Total numbers of Iba1 positive activated and silent (based on morphological appearance) cells were estimated using the optical fractionator method(3) (Strereo Investigator software; MicroBrightField Inc., USA). The counting criterion was the soma of the Iba1 positive cell. Statistical analysis was performed in GraphPad Prism 7.0 using One-Way ANOVA and Tukey post-hoc test for data that passed normality test and Kruskal-Wallis with Dunn's post-hoc test for non-parametric data.

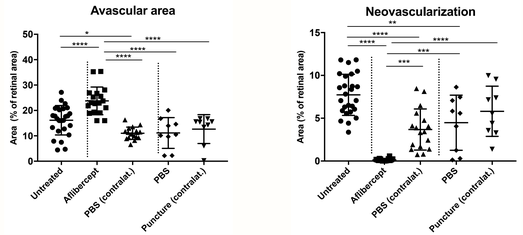
Commercial relationships
Experimentica Ltd: Maria Vähätupa, Jenni J. Hakkarainen, Simon Kaja, Giedrius Kalesnykas
K&P Scientific LLC: Simon Kaja

Microkatu 1
P. O. Box 1199
70211 Kuopio, Finland
info@experimentica.com
www.experimentica.com

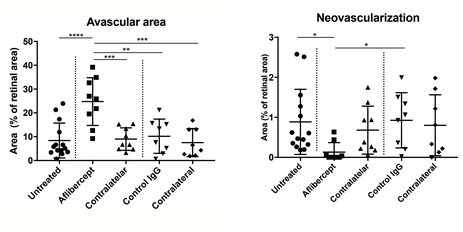
Results

Mouse OIR





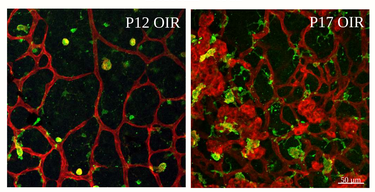
Rat OIR



Aflibercept increased the size of AVAs (outlined in yellow) by 47% (P<0.0001) and inhibited NV (white arrows) by 98% (P<0.0001) in mice compared to untreated control retinas. PBS injection decreased AVAs by 31% (P=0.0192) and reduced NV by 42% (P=0.004) compared to untreated controls. Puncture through the sclera had a similar effect as PBS, but the difference did not reach statistical significance (P>0.05).

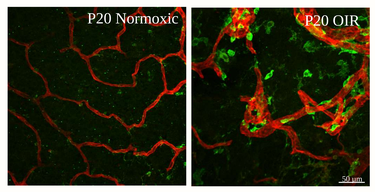
Microgliosis in Mouse OIR model

Isolectin + Iba1



Microgliosis in Rat OIR model

Isolectin + Iba1



Total number of microglia (Iba1 positive cells, in green) was increased in rat OIR model (at P20, P=0.028). A trend towards increased total number of microglia was observed in mouse OIR model (at P17, P=0.069). However, statistically significant differences were not observed between treatment groups from either of the models.

The rat OIR model showed a less severe phenotype than the mouse OIR model with 43% of retinas showing AVAs (outlined in yellow) of <5% and 71% of retinas showing NVs of <1% (white arrows). Aflibercept increased the size of AVAs by 213% (P<0.0001) and inhibited NV by 82% (P=0.012) in rats compared to untreated controls. Aflibercept treated eyes also had small AVAs around the optic nerve head. Administration of control IgG did not have any significant effect neither to AVAs nor to NV in the rat model as compared to aflibercept treated eyes (P>0.05).

Conclusion

The mouse OIR model produced more pronounced pathological changes compared to the rat model. Aflibercept blocked physiological revascularization and pathological neovascularization in both models. The robustness of the response makes the mouse OIR model particularly well-suited for efficacy pharmacology studies targeting neovascular processes. It should also be kept in mind that sham injection or puncture alone can lead to significant effects in the mouse OIR model using intravitreal administration. In rats this effect was not observed , possibly due to a less severe pathology, or a bigger vitreous volume, in proportion to the injection volume.

References

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