THE ROLE OF LOX AND LOXL2 IN INFLAMMATION AND FIBROSIS IN A LASER INDUCES MOUSE MODEL

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BACKGROUND AND AIM

Age related macular degeneration (ARMD) is the major cause of irreversible blindness worldwide. Abnormal growth of choroidal blood vessels through the Bruch's membrane into the subretinal space (CNV) leads to inflammation, angiogenesis and finally fibrosis in the macula. LOX (lysyl oxidase) and LOXL (lysyl oxidase like proteins: LOXL1-2-3-4) are enzymes that are associated with the cross linking of elastin and collagen, leading to an increase of fibrosis (1). There is growing evidence that these molecules also play a role in neovascularisation. LOX is associated with proliferative diabetic retinopathy and retinal detachment (2). LOXL1 polymorphisms are known to be associated with pseudoexfoliation glaucoma (3). A potential advantage of anti-LOX(L) administration would be the effect on inflammation and fibrosis in the pathogenesis of ARMD.

The aim of this project is to investigate:

1. The expression of LOX and LOXL2 in a mouse model for CNV, and its association with angiogenesis, inflammation and fibrosis in this model.
2. The efficacy of anti-LOX and/or anti-LOXL therapy in a mouse model of CNV.

MATERIAL AND METHODS

At first, we would like to investigate the role of LOX and LOXL on angiogenesis, inflammation and fibrosis after laser photocoagulation. CNV will be induced in C57 Bl/6 mice, 8-10 weeks old. Three laser burns will be placed with a green laser (532nm) at 9, 12, and 3 o'clock positions in each retina. Each spot will be set with a 50 (m spot size, 0.05 second duration and energy of 400mW. In order to check the effect of LOX and LOXL on fibrosis and inflammation in the disease progress, mice will be killed on different time points. 2, 4, 7, 14, 28 and 35 days after laser mice will be sacrificed and immediately after death, both eyes will be enucleated. In one eye the expression of LOX and LOXL will be checked by using real-time reverse transcriptase polymerase chain reaction (RT-PCR). The other eye of each animal will be used for different stainings. Seven-μm thin slides will be stained for Sirius Red and Trichrome to check fibrosis. Angiogenesis and inflammation will be investigated by respectively an immunohistochemical staining for CD31 and CD45. The expression of LOX(L) will be checked by an immunohistochemical staining by using a anti-LOX(L) polyclonal antibody. Finally, we would like to check the anti-angiogenic, anti-inflammatory and anti-fibrotic efficacy of anti-LOX and anti-LOXL2 antibody in the pathogenesis of AMD. CNV will be induced in mice as described above. Two daily injection with LOX(L) antibodies or control solution (PBS-Tween 20%) will be given intraperitoneally from day 0 after lasering until day 34. Mice will be sacrificed 35 days after lasering. Immediately after death, both eyes will be enucleated. One eye will be used as a control to check RNA-expression of LOX and LOXL. The other eye will be used to perform different immunohistochemical stainings (HE, Sirius Red, Trichrome, CD31, CD45 and LOX(L)).

CONCLUSION

Our proposed research will elucidate the efficacy of anti-LOX and/or anti-LOXL therapy in CNV and will highlight any anti-angiogenic, anti-inflammatory, and/or anti-fibrotic effects.

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