Kyung Ho Park[1], Hyun Woo Park[1], Hee Young Lee[1], Yeongyeong Kim[1], Ajin Lee[1], Ah Hyun Lee[1], Hyuna Kim[1], Larry C. Park[1]

[1]Naason Science Inc.

Validation of the two-stage laser mouse model of subretinal fibrosis and the therapeutic effect of aflibercept

Purpose:

Subretinal fibrosis often accompanies the neovascular form of age-related macular degeneration and leads to photoreceptor damage and serious vision loss even with therapy. We aimed to validate a model of subretinal fibrosis using two-stage laser burns and evaluate the effect of aflibercept (Eylea®), an inhibitor of vascular endothelial growth factor, in this model.

Methods:

Three burns were applied by a 532 nm laser (200 mW, 100 ms, 50 µm) in each eye of male C57BL/6 mice at 7 weeks of age. 7 days later, three additional burns using the same laser settings were applied on the same positions as the initial burns. 10, 20, 30, and 40 days after the second laser burns, posterior eyecups were flat-mounted, and the neovascular and fibrotic lesions were stained with isolectin B4 (IB4) and collagen 1 (Col1). The results at each timepoint were compared with lesions induced using single laser burns (4 mice/timepoint/model).

To test the effect of aflibercept, a separate batch of mice were injected intravitreally with aflibercept (2 mg/ml) or PBS immediately after the second laser (8 mice/group). Flatmount samples were collected 10 days after the second laser for staining analysis of IB4 and Col1. Student's t-test was used for statistical analysis.

Results:

The two-stage laser model resulted in larger IB4 and Col1 staining areas compared with the single laser model at 10 (IB4: 85.4% larger in two-stage laser model, p=0.056; Col1: 89.4%, p=0.077), 30 (IB4: 95.9%, p=0.013; Col1: 96.7%, p<0.001), and 40 days (IB4: 62.8%, p=0.16; Col1: 67.2%, p=0.082) after the second laser. At 20 days after the laser, staining area of the two-stage laser lesion was lower compared with the other timepoints and comparable to single-laser lesions (IB4: -1.64%, p=0.95; Col1: -35.5%, p=0.18).

There was no significant difference in staining area of IB4 (aflibercept: 67100±6070 µm^2; PBS: 66300±8310 µm^2, p=0.94) and Col1 (aflibercept: 67500±6400 µm^2; PBS: 71400±7090 µm^2, p=0.69) between the groups treated with aflibercept and PBS 10 days after the second laser.

Conclusion:

The two-stage laser mouse model produced larger fibrosis lesions compared with the single laser model. On the other hand, treatment with aflibercept did not significantly reduce subretinal fibrosis induced by the two-stage laser model. We intend to expand our investigation of subretinal fibrosis to porcine models and other targets for treatment.