Targeting plasma kallikrein with a novel bicyclic peptide inhibitor (THR-149) reduces retinal inflammation and reactive gliosis in a diabetic rat model

Tine Van Bergen [1], Isabelle Etienne [1], Tjing-Tjing Hu [1], Lies De Groef [2], Lieve Moons [2], Jean H.M. Feyen [1], Alan Stitt [1]

[1] Oxurion NV, Gaston Geenslaan 1, 3001 Heverlee, Belgium

[2] KU Leuven, Neural Circuit Development & Regeneration Research Group, Department of Biology and Leuven Brain Institute, Leuven, Belgium

PURPOSE (200 words). Anti-VEGF therapies are the current mainstay treatment for diabetic macular edema (DME), although it is now established that a significant number of patients respond sub-optimally or not at all to VEGF inhibition. Alternative therapeutic strategies for anti-VEGF treatment for DME are therefore urgently needed. Elevated plasma kallikrein (PKal) is a known pathogenic factor in DME where it drives VEGF-independent retinal inflammatory processes and vasopermeability. In the current study, we determined the potential of THR-149, a novel potent and highly specific peptide inhibitor for PKal, to prevent key pathologies associated with DME in diabetic rats, especially in relation to elements of the neurovascular unit, such as retinal inflammation and loss of normal Müller cell homeostatic function.

SETTING/VENUE (100 words). The efficacy of intravitreal (IVT) administration of THR-149 was evaluated in a diabetic streptozotocin (STZ)-induced rat model, in which the impact on glial and immune cell function in the diabetic retina was explored. This preclinical study was conducted at Oxurion, NV (Leuven, Belgium).

METHODS (200 words). Following STZ-induced diabetes in the rat, THR-149 (12.5 µg/eye) and its vehicle was administered in both eyes either via a single or via 3 consecutive IVT injections (with 1-week interval, n=7 rats/group). Untreated, non-diabetic rats served as a control (n=5 rats). At 4 weeks post-diabetes, the effect of all groups was compared by histological analysis of the retina for Iba1-positive immune cells, vimentin-positive Müller cells, potassium- and water homeostasis-related channels (Kir4.1 and AQP4, respectively) at the glio-vascular interface. The Iba1-, vimentin- and Kir4.1-positive area in the retina was investigated by measuring the ratio of the immuno-positive area over the retinal area per image, whereas the AQP4-positive area was measured over the ONL area and expressed as percentage. Iba1-positive cells were also counted, and further classified as activated or non-activated cells, based on their morphology. Statistical analysis was performed with a one-way analysis of variance using a Bonferroni multiple comparison test.

RESULTS (200 words). Analysis of the Iba1 staining in the retina following 4 weeks of diabetes showed a significant increase in the Iba-1 positive area and cell number when compared to non-diabetic controls ($p<0.05$). Single and repeated administration of THR-149

reduced the inflammatory positive area ($p<0.05$) when compared to vehicle, as well as the total number and activation state of immune cells, a key readout of retinal inflammation. Repeated administration of THR-149 also reduced the diabetes-induced increase of vimentin-positive cells (reactive gliosis) at 4 weeks after diabetes onset ($p<0.05$) versus vehicle, whereas no significant difference following a single administration of THR-149 was seen ($p=0.99$) versus vehicle. At the molecular level, reduced Kir4.1-channel levels in the diabetic retina were restored to control non-diabetic levels in the presence of repeated THR-149 ($p<0.01$) compared to vehicle. In contrast, little to no effects were observed on the diabetes-induced AQP4-channel levels by THR-149.

CONCLUSIONS (200 words). These data demonstrate that repeated administration of THR-149, a novel bicyclic peptide inhibitor of PKal, reduced several DME-related key pathologies, such as activation of retinal microglia/macrophages and Müller cells in the diabetic rat retina and restored the reduced expression of Müller cell Kir4.1-positive channels. These observations indicate that modulation of the PKal-pathway using THR-149 has clinical potential to treat patients with DME and that potentially repeated IVT injections are needed to achieve a more complete therapeutic effect in elements of the neurovascular unit.