Quantitative assessment of retinal permeability in the VEGF-induced mouse model: Validation using VEGF-Trap and a novel integrin antagonist THR-687

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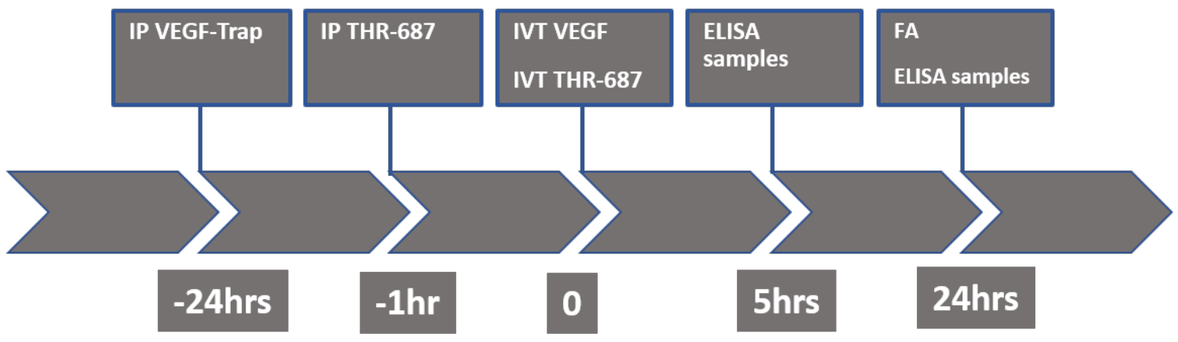
Abstract

Diabetic retinopathy (DR), the most common microvascular complication of diabetes mellitus, is the leading cause of visual impairment in the working-age population of the Western world. In this study, we validated the vascular endothelial growth factor (VEGF)-induced retinal permeability model in mice. This model is especially suitable to investigate vascular complications that are also implicated in DR pathogenesis. Intravitreal injections of pathophysiological concentrations of VEGF can induce DR-like vascular dysfunctions such as vascular tortuosity, micro-aneurysms and vascular leakage [1]. Two different analytical methods to determine and quantify retinal leakage in the mouse eye were evaluated, more specifically the non-invasive fluorescein angiography and the detection of albumin levels in retinal homogenates through albumin ELISA. In addition, the anti-leakage effect of THR-687, which is a novel small molecule integrin antagonist, was also assessed in the model. It is well documented that integrins are associated with various pathological DR hallmarks, such as neovascularization, inflammation and vascular leakage [2].

Material and Methods

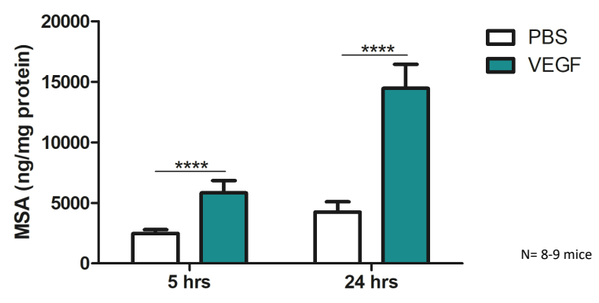
Induction of VEGF-induced hyperpermeability: Vascular leakage was induced in 8- to 10-week-old male C57Bl6/J mice via intravitreal (IVT) injection of recombinant human VEGF (300ng, 1µL, R&D Systems).
Fluorescein angiography (FA): FA images were captured using Heidelberg Spectralis every minute up to 12 minutes after subcutaneous injection of 100µL of 5% (w/v) sodium fluorescein (SERB). Retinal leakage was defined as the increase in fluorescent area over time and quantitatively analyzed via Zen software (Zeiss).
Mouse serum albumin (MSA) ELISA: After transcardial perfusion of the retinal vasculature (10U/mL heparin in 0.9% (w/v) NaCl), retinas were dissected and MSA ELISA (Bioké) was performed on retinal homogenates.
Compound administration: The efficacy of THR-687 was evaluated via intravitreal (IVT, 50µg) and/or intraperitoneal (IP, 15mg/kg) administration routes. VEGF-Trap (Aflibercept, IP, 25mg/kg) and phosphate-buffered saline (PBS) were included as positive and negative control, respectively (see scheme).
Data analysis: Data were analyzed via one- or two-way ANOVA using Graphpad Software, and shown as average ± SEM.



Results

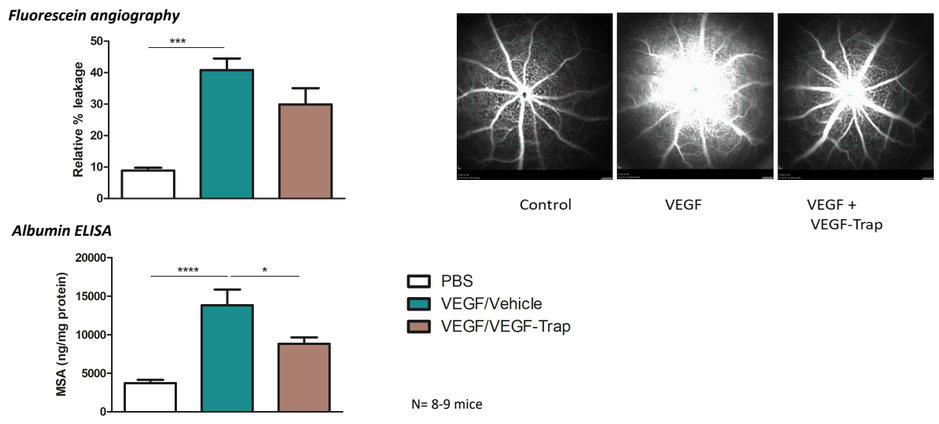
Time-course of retinal hyperpermeability after IVT administration of VEGF

A significant increase in MSA levels was observed at 5 hours post-injection (hpi) (2.4-fold), however, retinal hyperpermeability was more pronounced at 24hpi (3.5-fold).



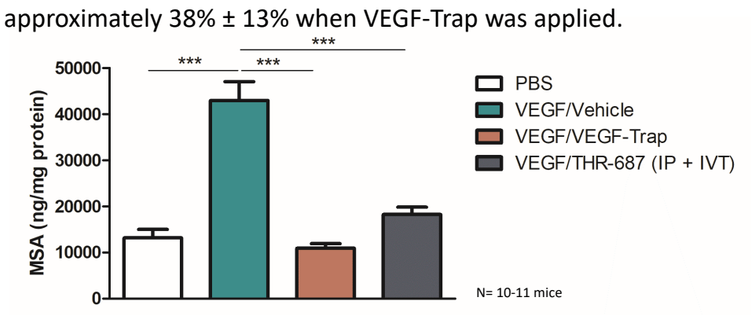
Comparison of different read-out methods to quantify the leakage induced by VEGF in the mouse retina

FA and MSA ELISA were quantitatively analyzed and showed a similar significant increase in vascular leakage after IVT administration of VEGF (24hpi), as well as a comparable reduction in leakage after treatment with the VEGF inhibitor VEGF-Trap (25mg/kg).



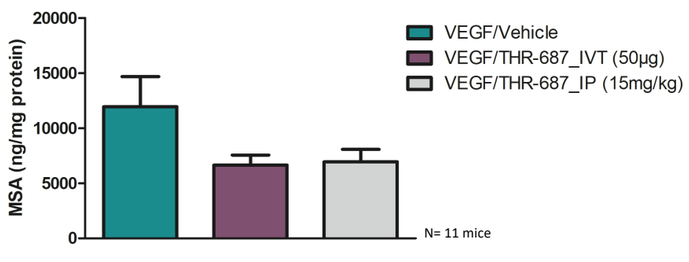
Combined administration of THR-687 in the VEGF-induced permeability model

Repeated experiments showed that combined IVT (50µg) and IP (15mg/kg) administration of THR-687 potently inhibited VEGF-induced retinal permeability by 43% ± 7%. Retinal leakage was reduced by approximately 38% ± 13% when VEGF-Trap was applied.



Comparison of different administration routes of THR-687 in the VEGF-induced permeability model

At 24hpi, a trend towards inhibition of the VEGF-induced retinal permeability was observed when THR-687 was administered either IVT or IP.



Conclusion

Preclinical experiments demonstrate that albumin ELISA can serve as an effective alternative to the non-invasive fluorescein angiography read-out. By using albumin ELISA, the VEGF-induced retinal permeability model proved to be a reproducible, sensitive and quantitative model that can be implemented to evaluate the anti-leakage properties of compounds. THR-687 was able to reduce the permeability in this model to the same level as VEGF-Trap, making it a promising next-generation therapy for DR.

References

[1] *Reyns et al. Selection Strategy of In Vivo Models for Ophthalmic Drug Development in Diabetic Retinopathy. J. Mol. Genet. Med. 2016;10:202*
[2] *Hu et al. The Potent Small Molecule Integrin Antagonist THR-687 is a Promising Next-generation Therapy for Retinal Vascular Disorders. Exp. Eye Res. 2019;180:43-52.*

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