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Poster 33

THR-687, a potent small molecule integrin antagonist, holds promise as a therapeutic approach for back-of-the-eye vascular pathologies

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Purpose

Pathologic neovascularization and vessel leakage are key drivers for vision loss in several back-of-the-eye diseases, such as diabetic retinopathy and age-related macular degeneration. In the eye, integrin receptors play an important role in (pathological) angiogenesis and vascular leakage. Blocking integrin receptors has the potential to inhibit these processes, independent of anti-VEGF responsiveness. In this study, we evaluated the integrin-blocking and anti-angiogenic properties of THR-687, a novel small molecule integrin antagonist.

Methods

Integrin profiling: The ability of THR-687 to antagonize various integrin receptors was studied via competition ELISA. 96-well plates were coated with an integrin receptor, an appropriate ligand (e.g. fibronectin) was then added to the wells in the presence of increasing concentrations of THR-687, and after 2h, the bound ligand was detected via Streptavidin-HRP and o-Phenylenediamine.

Oris™ cell migration assay: Human umbilical vein endothelial cells (HUVEC) were seeded onto fibronectin-coated Oris™ 96-well plates. After adherence, the stoppers were removed, creating circular cell-free areas, and cells were allowed to migrate into the detection zone in the presence of THR-687 at the indicated concentration range. After 24h, the amount of fluorescently labeled cells in the detection zone was measured using a microplate reader.

Mouse choroidal explant model: RPE-choroid-sclera explants from 4-week-old C57Bl/6J mice were embedded in a 3D fibrinogen/fibrin gel in 48-well plates. Addition of serum-containing medium induces extensive endothelial cell sprout outgrowth into the matrix. The effect of increasing concentrations of THR-687 on vessel sprouting was evaluated by manual analysis on bright field images after 3 – 4 days in culture.

Cynomolgus choroidal neovascularization (CNV) model: The anti-angiogenic potential of 3 different dose levels of THR-687 (4.5mg/eye (n = 6), 2.25mg/eye (n = 5) and 0.45mg/eye (n = 6)) were investigated in the cynomolgus laser-induced CNV model, study performed by Charles River Laboratories. Each experimental group received 3 intravitreal (IVT) injections of THR-687 or its formulation buffer (n = 6) with a weekly interval (Day 1, 8 and 15). Fluorescein angiography was performed to assess the grade of CNV-induced leakage in the laser spots on Day 1 (baseline, before IVT treatment), and toward the end of Week 2 and Week 3 after the start of treatment.

Results & Discussion

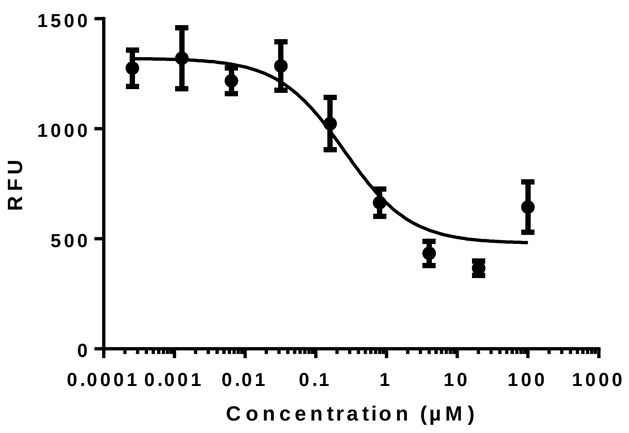
1. THR-687 potently inhibits integrin receptors involved in angiogenesis and permeability

The ability of THR-687 to bind to various integrins of human origin, and to compete with the binding of their natural ligands, was assessed via **competition ELISA**. THR-687 inhibited multiple integrin receptors belonging to the RGD class with IC_{50} values in the low nanomolar range, as shown in the table below (average ± SD, n≥ 3).

Human integrins, IC_{50} values									
RGD binding (nM)							Collagen binding (µM)	Laminin binding (µM)	Leukocyte specific (µM)
$\alpha_V\beta_1$	$\alpha_V\beta_3$	$\alpha_V\beta_5$	$\alpha_V\beta_6$	$\alpha_V\beta_8$	$\alpha_5\beta_1$	$\alpha_{IIb}\beta_3$	$\alpha_2\beta_1$	$\alpha_3\beta_1$	$\alpha_4\beta_1$
3.2 ± 1.3	4.4 ± 2.7	1.3 ± 0.5	9.0 ± 5.3	1.5 ± 0.7	6.8 ± 3.2	105 ± 26	121 ± 25	> 5,000	3.8 ± 1.7

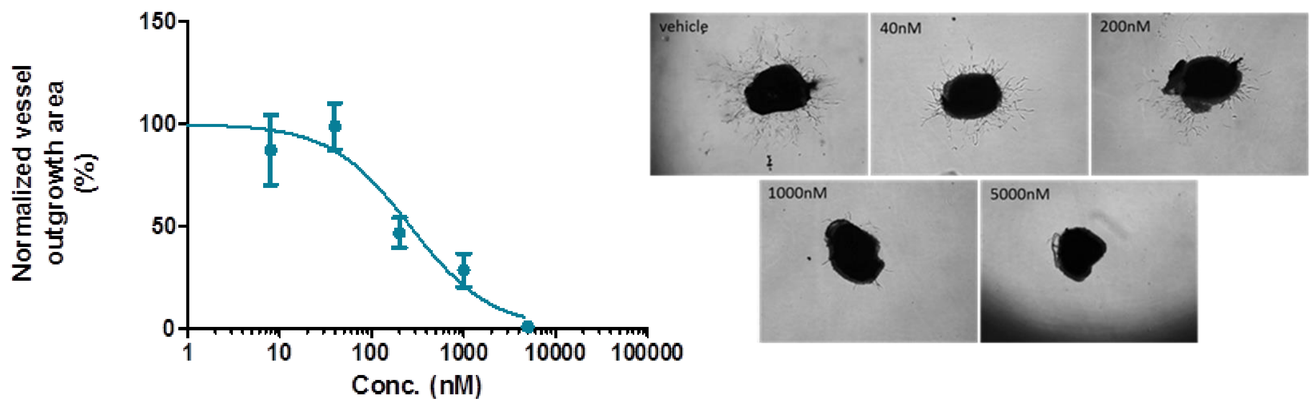
2. THR-687 inhibits endothelial cell migration

Integrins play a crucial role in endothelial cell migration and adhesion. The inhibitory effect of THR-687 on HUVEC migration was investigated in the Oris™ cell migration assay. THR-687 potently inhibited HUVEC migration with an IC_{50} of 258 ± 65nM. Data shown below are average values ± SEM of at least 3 independent experiments.



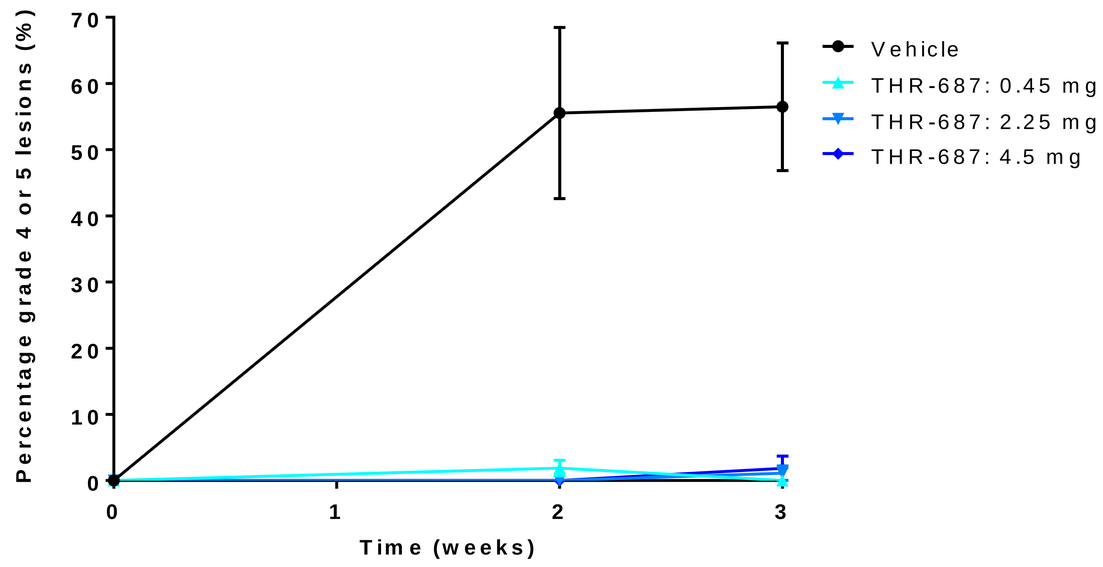
3. THR-687 inhibits blood vessel outgrowth from *ex vivo* murine choroidal explants

As integrins are highly involved in (pathologic) angiogenesis, the anti-angiogenic potential of THR-687 was evaluated in a mouse microvascular explant model, which closely mimics *in vivo* choroidal angiogenesis. As shown in the representative graph below, THR-687 dose-dependently inhibited choroidal vessel outgrowth *ex vivo* with an IC_{50} of 0.2 ± 0.086µM. Representative pictures of all concentrations are shown below. Data are average values ± SEM of at least 3 independent experiments.



4. THR-687 potently inhibits choroidal neovascularization in the monkey CNV model

It has been described that integrin antagonists can inhibit laser-induced CNV in a monkey model. Therefore, the efficacy of different doses of THR-687 was investigated. All tested dose levels of THR-687 potently inhibited angiogenesis-induced leakage in the cynomolgus CNV model.



5. Pharmacology studies indicate a good safety profile for THR-687

	STUDY	FAVORABLE SAFETY OUTCOME
Safety Pharmacology	Safety assessment on the rat central nervous system (CNS)	THR-687 dose levels of up to 15mg/kg had no effect on the CNS
	Cardiovascular and respiratory assessment in mini-pig	THR-687 is not cardiotoxic at dose levels up to 20mg/kg
	Tolerability of multiple IVT injections in rabbits	Repeated administration of 3mg/eye of THR-687 is a tolerable dose in rabbits
Toxicology	Toxicity study of a single IVT & IV injection in rabbits	THR-687 IVT dose levels up to 5mg/eye & IV dose levels up to 5 mg/animal were tolerable in rabbits
	Toxicity study of a single IVT & IV injection in mini-pigs	THR-687 IVT dose levels up to 10mg/eye & IV dose levels up to 10 mg/animal were tolerable in mini-pigs
Genotoxicity	Bacterial reverse mutation assay	THR-687 was negative in the bacterial reverse mutation assay
	In vitro mammalian cell micronucleus screening	THR-687 was negative in the in vitro mammalian micronucleus screening assay
	In vivo micronucleus and Comet assay	THR-687 did not induce significant toxic/cytotoxic effects nor genotoxic effects

Conclusion

THR-687 exhibited potent anti-angiogenic activity in *in vitro* assays and strongly inhibited CNV-induced leakage in a monkey CNV model. Extensive toxicology and safety pharmacology studies indicated a good safety profile of THR-687. Thus, THR-687 is a promising drug candidate for the treatment of vision-threatening retinal pathologies such as DR and wet AMD.

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