Introduction

Ocriplasmin is a non-surgical treatment for vitreo-macular adhesion/traction (VMA/VMT). It is proposed that ocriplasmin enzymatically cleaves extracellular matrix proteins at the vitreo-retinal interface, hereby inducing posterior vitreous detachment (PVD) and resolving traction. Ocriplasmin was shown to be efficacious for treatment of VMT with or without full thickness macular hole (<400µm) in several clinical studies.

To gain preclinical insight into the mechanism of action of ocriplasmin, we evaluated vitreo-retinal tissues from an in vivo porcine PVD model and in vitro retinal cell models.

Methods

Porcine PVD model samples: Information on the porcine PVD model is available on poster 4042. Vitreo-retinal tissues were collected at sacrifice, at time points up to 6 weeks post-injection.

Histology: Eyes were fixed in 4% PFA, after overnight storage in PBS with 3% sucrose the eyes were slowly dehydrated in an increasing ethanol dilution series. Deparaffinized sections were stained with either hematoxylin and eosin or antibodies specific for pan-laminin, fibronectin and collagen IV.

Vitreous cytokine profile: Detection of CCL-2 was performed using a custom ELISA assay. Detection of the remaining cytokines was performed using MILLIPLEX® MAP technology. Untreated eyes as well as lipopolysaccharides treated eyes (100 ng/eye) were included as controls.

Transepithelial/endothelial resistance (TEER): Primary human retinal microvascular endothelial cells (HRMEC, Cell-systems) and human retinal pigment epithelium cells (ARPE-19, ATCC) were cultured according to supplier instructions. TEER was recorded in a CellZscope 24 well module (nanoAnalytics).

Results & Discussion

1. Ocriplasmin-induced PVD does not significantly alter distribution of retinal extracellular matrix proteins.

Vitreo-retinal tissues from the porcine PVD model were stained for laminin, fibronectin and collagen IV (here depicted 6 weeks post-injection).

At the vitreo-retinal interface, ocriplasmin-induced PVD could be clearly visualized. In agreement with its proposed mechanism of action, ocriplasmin segregated extracellular matrix proteins at the inner limiting membrane into two layers. One layer remained attached to the inner retinal surface, and the other migrated with the PVD interface into the vitreous. Intra-retinal presence and distribution of laminin and collagen IV (localizing at retinal blood vessels) did not appear altered in ocriplasmin versus control eyes. Morphology of the retina and retinal layers was not altered compared to control eyes (see also poster 4042).

2. Ocriplasmin-induced PVD does not correlate with an acute inflammatory response.

14 markers of inflammation were measured in vitreous samples of pigs treated with ocriplasmin or vehicle. No statistically significant differences were found between ocriplasmin versus vehicle-treated eyes. No correlation was found between subretinal lucencies or vitreal hyper-reflective spots and an inflammatory signature. Overall, all markers remained in the range of non-injected eyes and no LPS-like acute inflammatory responses were observed. Only a minor and transient increase of inflammation markers was observed in all eyes, irrespective of treatment, most likely related to intravitreal injection.

3. Only high ocriplasmin doses can induce endothelial, but not epithelial permeability in vitro. All effects are rapidly and fully reversible.

Ocriplasmin did not induce permeability in ARPE19 cells and only from 6µg/mL in HRMEC cells. All observed TEER effects were fully and rapidly reversible upon treatment wash-out (W). No effects were observed on cell adhesion (Capacitance). Inactive ocriplasmin was unable to induce permeability. Further study is required to assess these in vitro observations in vivo.

Conclusions

Closer examination of vitreo-retinal tissues originating from a porcine ocriplasmin-induced PVD model (see poster 4042) did not indicate retinal changes, retinal extracellular matrix redistribution nor strong acute inflammatory signatures. In vitro effects on endothelial barrier permeability were rapidly and fully reversible. Their in vivo relevance needs to be assessed.