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Posterior Vitreous Detachment: more a case of the fibronectin interface than the inner limiting membrane?

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Introduction

The separation of the posterior vitreous cortex and the inner limiting membrane (ILM) of the retina, called Posterior Vitreous Detachment (PVD) can be detected by Optical Coherence Tomography (OCT). To visualize the distributions of the inner limiting membrane components [1] on eyes presenting enzymatically induced PVD, we performed and analyzed different histological stains. The impact of V20I on different ILM components were also studied *in vitro*.

Methods

Lysis profiles of fluoresenctly labeled substrates in presence of V20I (a microplasmin analogue able to induce PVD) were studied *in vitro*. The test was performed in a 96-well micro titer plate that was coated with the fluorescently labeled substrates. Various dilutions of V20I were tested after which the plate was covered and incubated at 37°C. Samples were analysed after different times (up to 3 hours).

Periodic acid shift (PAS) and immunohistochemical stains were performed to visualize the distribution of the ILM components on porcine and murine eyes presenting PVD (*vs.* control eyes) induced by a 29 µg/mL V20I intravitreal injection (up to 6 weeks follow up). After fixation of the eyes in formaldehyde 4%, dehydration was achieved by immersion in increasing concentrations of alcohol. Following dehydration, the eyes were incubated with xylene before being impregnated with paraffin wax. Transverse, serial sections (7 and 15 µm thick, for mouse and pig respectively) were cut. After deparaffinization and antigen retrieval stage, slides were incubated with selected antibodies. Amplification stages were performed before fluorescence visualization of the specified marker. Among the marker stained were collagen IV, laminin alpha 1 and fibronectin. Periodic acid shift staining was performed on adjacent cross sections to assess potential structural change, including PVD.

Results

1. *In vitro* studies

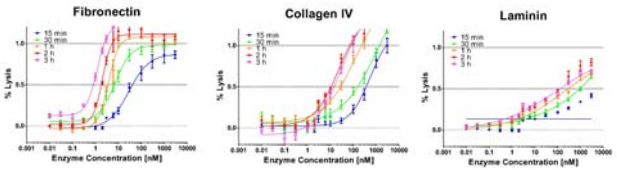

Figure 1 : V20I-mediated lysis of fibronectin, collagen IV and laminin. Fibronectin appears to be a preferential substrate for V20I with a IC_{50} (after 3 hours) equal to 1.6 nM (compared to a IC_{50} equal to 10.6 nM for collagen IV and 361 nM for laminin).

2. Mouse eye histological stains

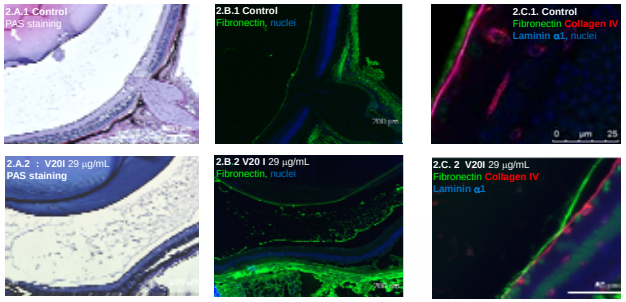

Figure 2 : **P**eriodic **A**cid **S**hift **PAS** (A) and immunohistochemistry stains (B and C) were performed on cross sections of mice eyes intravitreally injected with vehicle (figures 2.A.1; 2.B.1, 2.C.1) or V20I (29 µg/mL, figures 2.A.2; 2.B.2, 2.C.2). PVD area could be detected by periodic acid shift and fibronectin stains, this on V20I intravitreally injected eyes. Upon PVD, fibronectin staining completely migrated with the PVD interface, cleanly detaching from the retinal cell layers.

3. Pig eye histological stains

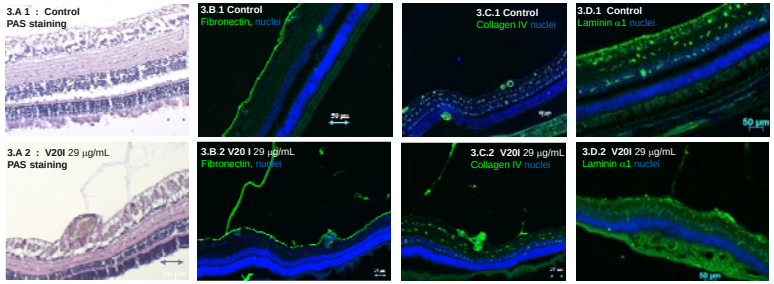

Figure 3 : **P**eriodic **A**cid **S**hift **PAS** (A) and immunohistochemistry stains (B,C and D) were performed on cross sections of pig eyes intravitreally injected with vehicle (figures 3.A.1; 3.B.1, 3.C.1, 3.D.1) or V20I (29 µg/mL, figures 3.A.2; 3.B.2, 3.C.2, 3.D.2). Upon PVD, fibronectin, collagen IV and laminin alpha1 do migrate with the PVD interface. However, staining is still detectable on the ILM for those markers.

Discussion

Different distribution patterns in the retina were observed for the selected markers. The inner retinal blood vessels were labelled by laminin apha 1 and collagen IV. Fibronectin, collagen IV and laminin alpha 1 were detected at the ILM. In the mouse model, segregation of the PVD interface could be assessed by PAS and fibronectin staining. In the pig model, fibronectin, collagen IV and laminin alpha 1 did migrate with the PVD interface. However, in the pig model, those markers were still present on the ILM, even in case of PVD. The difference obtained could be due to the size of the eyes and resulting force distribution on the ILM upon the traction generated by PVD.

Conclusions

Whilst collagen IV and fibronectin were substrates of V20I in *in vitro* test, those proteins were still detectable by immunohistochemistry in the mouse and pig after induction of PVD by V20I. In contrast to Chen *et al*. finding, we do conclude to a partial digestion of those proteins in *in vivo* situation [2]. The distribution patterns observed for collagen IV and fibronectin are similar in mouse and pig in normal control retina. Different behaviors of the studied substrates were observed between the pig and mouse after induction of PVD by V20I. The complete fibronectin segregation with the PVD interface seems to be specific to the mouse after PVD induction by V20I. The human situation remains to be investigated.

Bibliography :
1. Halfter *et al*, 2008. Origin and turnover of ECM proteins from the inner limiting membrane and vitreous body
2. Chen *et al*, 2009 : Microplasmin Degrades Fibronectin and Laminin at Vitreoretinal Interface and Outer Retina during Enzymatic Vitrectomy.