EFFECTS OF RIMONABANT ON PORCINE RETINAL MÜLLER GLIAL CELLS

Posterboard#: B0314

Abstract Number: 1951 - B0314

AuthorBlock: *Zhao-Hui Song[1], Kyle Funk[1], Lucy Sloan[1], Md.Istiaq Obaidi[2], Shigeo Tamiya[2]*
[1]Pharmacology and Toxicology, University of Louisville, Louisville, Kentucky, United States; [2]Ophthalmology and Visual Science, The Ohio State University, Columbus, Ohio, United States;

DisclosureBlock: Zhao-Hui Song, None; Kyle Funk, None; Lucy Sloan, None; Md.Istiaq Obaidi, None; Shigeo Tamiya, None;

Purpose
Retinal fibrosis associated with conditions such as proliferative vitreoretinopathy, age-related macular degeneration (AMD), and proliferative diabetic retinopathy is detrimental to vision. Fibrosis is considered an aberrant wound healing response marked by the development of collagen-rich scars. Retinal Müller glial (MG) cells contribute to several conditions resulting in retinal fibrotic scars. Upon exposure to TGF-β, a key fibrotic cytokine, MG cells trans-differentiate to myofibroblasts marked by the integration of α-SMA fibers into F-actin stress fibers which confers strong contractility. Myofibroblasts produce and contract the collagen-rich fibrotic scar and disrupt retinal architecture. In this study, we investigated the effects of rimonabant, a selective CB1 cannabinoid receptor antagonist, on TGF-β2 induced porcine MG cell contraction and α-SMA expression *in vitro*.

Methods
Porcine MG cells were isolated as previously described using papain dissociation kit. The collagen matrix contraction assay was used to assess myofibroblast function. Briefly, MG cells (10x10^4) were plated on solidified collagen in a 24-well plate and treated with rimonabant and TGF-β2 (10 ng/mL) for 72 hours. Collagen gels were released from the well, allowed to contract for 24 hours, and photographed for analysis. Collagen gels with attached cells were subsequently used for western blot analysis. For immunocytochemistry, collagen gels with cells attached were not released from the well, but rather fixed and stained for α-SMA, F-actin, and DAPI.

Results
Using an *in vitro* collagen matrix contraction assay, we found that rimonabant inhibited TGF-β2 induced contraction of collagen matrices by porcine MG cells. This effect was concentration-dependent, with significant inhibition of contraction at 3 and 10 μM. Immunocytochemistry showed that at 3 μM rimonabant significantly decreased expression of α-SMA fibers in stress fibers of the TGF-β2 stimulated porcine MG cells. Lastly, western blot analysis demonstrated that rimonabant downregulated protein expression of TGF-β2 induced α-SMA *in vitro*.

Conclusions
Taken together these results indicate that rimonabant has potential to inhibit TGF-β2-induced fibrosis in the retina. Further

studies are warranted to investigate the mechanism of action, other fibrotic end points, as well as potential in *in vivo* models of retinal fibrosis.