**Title:** The Effect of Ibudilast on Gliosis in Murine Retinal Detachment and Chick Excitotoxic Retinal Damage Models

**Introduction:** Retinal gliosis, an abnormal wound-healing response to retinal damage, can lead to excessive scare tissue formation, as seen in proliferative vitreoretinopathy (PVR) following retinal detachment (RD). Retinal gliosis also accompanies excitotoxic retinal damage, which is prevalent in retinal ischemic disease. Glial Fibrillary Acidic Protein (GFAP) levels are often used to assess gliosis. Our previous research shows that the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) is upregulated following RD and in N-methyl-D-Aspartate (NMDA) mediated excitotoxic damage. We measured GFAP protein expression to evaluate the effect of ibudilast, a MIF and phosphodiesterase inhibitor, on gliosis in murine RD and chick NMDA retinal damage models.

**Methods:** Experiments were performed under IACUC approved protocol. Chicks were treated with intravitreal injection of NMDA (500nmol/20ul) with or without ibudilast (1mg/ml) (n=6/group). Chick eyes were enucleated and fixed 1 day (D1) or 9 days (D9) after treatment to detect GFAP immunofluorescence. RDs were induced in mice by subretinal injection of hyaluronic acid (10 mg/ml) (n=6-7/group). Ibudilast (1mg/L) or vehicle control was given intraperitonially 24h prior to RD and continued daily until sacrifice at day 3 (D3) or 14 (D14). Mice eyes were enucleated, fixed, and stained for GFAP. Confocal microscopy was used for imaging. Analysis was performed using NIS Elements software. Statistics were performed using paired Student’s T-test for chick NMDA model and unpaired Student’s T-test for mice RD model with p-value ≤ 0.05 considered statistically significant.

**Results:** In chick NMDA retinal damage model, measurements of mean GFAP intensity were compared between paired NMDA damaged eyes with or without ibudilast. Ibudilast significantly reduced GFAP expression (NMDA: 42.80 ± 11.71 vs NMDA + ibudilast: 24.56 ± 9.957, p=0.0088) at D9. In murine RD model, the mean retinal GFAP intensity was compared between ibudilast and vehicle treated mice at D3 and D14. No significant difference was detected between groups at D3 and D14 (p=0.1302 and p=0.3284, respectively).

**Conclusions:** Decrease in GFAP expression following ibudilast treatment in chick NMDA retinal damage may indicate anti-gliotic function. Our research suggests ibudilast can be further investigated as a potential therapeutic strategy for retinal diseases with pathophysiology of excitotoxic damage.