OPEN ACCESS
ARVO Annual Meeting Abstract | June 2023

The Phospholipase D pathway modulates oxidative stress in retinal pigment epithelium cells exposed to high glucose levels

PAULA ESTEFANIA TENCONI; María Sol Echevarría; Norma María Giusto; Melina Valeria Mateos

+ Author Affiliations & Notes

Investigative Ophthalmology & Visual Science June 2023, Vol.64, 4475. doi:

Abstract

Purpose : Oxidative stress (OE) and inflammation are involved in the pathogenesis of several retinal diseases. We previously demonstrated that classical phospholipase D isoforms (PLD1 and 2) mediate the inflammatory response of retinal pigment epithelium (RPE) cells induced by high glucose (HG) levels. Furthermore, a significant increase in reactive oxygen species (ROS) was observed in RPE cells exposed to HG. The aim of the present work was to study the relationship between OE and PLD activation observed in HG- treated RPE cells.

Methods : RPE cells (ARPE-19 and D407) were exposed to HG (33 mM) or to normal glucose levels (NG, 5.5 mM) for 24 h. To inhibit classical PLDs cells were pre-incubated with 0.5 µM of VU0359595 (PLD1i) to inhibit PLD1 or 0.5 µM of VU0285655-1 (PLD2i) to inhibit PLD2 for 30 min at 37°C prior to cell exposure to HG. To inhibit cyclooxygenase-2 (COX-2) 10 µM of celecoxib was used. Inhibitors were also present during HG treatment. ROS production was assessed using the probe DCDCDHF. Immunocytochemistry assays (ICC) and western blots were performed to evaluate nuclear factor erythroid 2–related factor2 (Nrf-2) pathway. Data were analyzed by ANOVA followed by Bonferroni's test, $p \leq 0.05$ were considered statistically significant.

Results : HG-exposure increased ROS levels (148%, $p < 0.0001$) in RPE cells with respect to NG. When cells were exposed to HG and incubated with PLD1i and PLD2i ROS generation was completely prevented. On the contrary, the inhibition of COX-2 was not able to prevent OE induced by HG. ICC showed Nrf-2 nuclear translocation in cells

exposed to HG and this effect was not observed when cells were treated with PLD1i and PLD2i. Nrf-2 activation correlated with and increased heme oxygenase-1 (HO-1) and superoxide dismutase-1 (SOD-1) expression in HG-exposed cells (by 42 and 43 % respectively, $p < 0.05$) but no differences were observed in cells treated with PLD1i or PLD2i with respect to NG.

Conclusions : Our previous findings together with results presented herein, demonstrate that PLD1 and PLD2 inhibition not only prevents the inflammatory response of RPE cells, but also decreases OE generated in RPE cells exposed to HG in a Nrf-2 and COX-2 independent manner. Further experiments are needed to fully elucidate the mechanisms by which the PLD pathway mediates OE in RPE cells exposed to inflammatory injury.

This abstract was presented at the 2023 ARVO Annual Meeting, held in New Orleans, LA, April 23-27, 2023.