Inositol 1,4,5-triphosphate receptor type 1 plays important role in retinitis pigmentosa, and its pharmacological blocking disorganizes inflammatory response

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ABSTRACT

Introduction: Retinitis pigmentosa (RP) is a disease that leads to a progressive loss of vision. Although the rod cell death pathway is well elucidaded, the process that leads to cone cell death is not well known, hypotheses include an involvement of cytoplasmic and mitochondrial calcium excitotoxicity. Therefore, type 1 inositol-1,4,5-triphosphate receptor (IP3R1), a well-described calcium receptor widely expressed in neuronal cells and responsible for intracellular calcium homeostasis, might influence the progression of the disease.

Objective: The aim of this study was to analyze the IP3R1 receptor distribution in the retina during RP progression.

Methods: C3H/HeJ and C57/Bl6 mice aging 0, 7, 14 and 25 days-post natal (P0, P7, P14 & P25) of both sexes were provided by ICB and UFABC vivarium, respectively, and maintained in 12:12h dark-light cycle. The animals were euthanized using intraperitoneal urethane (25%) and posterior decapitation. Retinas were harvested for western blotting (WB, n=5), and immunofluorescence (IF, n=6). For IP3R1-DAPI colocalization, we employed Manders' coefficient analyses. IP3R1 antagonist, 2-APB bloker, was delivery via subretinal injections. Cytokine levels was measure Multiplex assay. All procedures were approved by CEUA/UFABC Committee (#9432030818) and the results were evaluated with t-test and two-way ANOVA followed by Sidak's post-hoc comparison.

Results: WB analysis showed difference in IP3R1 protein levels comparing C57/Bl6 and C3H/HeJ at P7 (0.28±0.07 vs. 0.81±0.02, respectively t-test, P=0.0013). The IF experiments revealed preferential IP3R1 localization in photoreceptors' segments and plexiform layers. When the IP3R1 cellular localization was analyzed, our results indicate that IP3R1 possibly translocates to perinuclear region during the progression of RP. Intensity analysis showed increased mean in the outer nuclear layer (ONL) of C3H/HeJ (1.40±0.16 vs 3.44±0.23 vs. 5.84±1.19 vs. 13.45±2.13, P0 vs. P7 vs. P14 vs. P25, P<0.0001), while Manders' analyses showed no differences in IP3R1-DAPI colocalization (P>0,05). The functional analysis after 2-APB injection showed no difference in ONL thickness. Multiplex cytokines analysis demonstrated disruption of the inflammatory response by decreasing the IL-10 (14.7±1.78 vs 6.21±3.51, Ctl vs 2-APB, P=0.02), IL-13 (7.75±1.12 vs 3.38±1.82, Ctl vs 2-APB, P=0.03) and IL-17 (0.82±0.07 vs 0.37±0.20, Ctl vs 2-APB, P=0.02), and TNFa (1.17±0,14 vs 0,51±0.28, Ctl vs 2-APB, P=0.02).

Conclusion: Our results revealed that IP3R1 translocates to the nucleus in RP progression and possibly has an important role in the neurodegeneration. Pharmacological blocking of IP3R1 disrupts inflammatory response.

KEYWORDS: Retinitis Pigmentosa; Neuroinflammation; Neurodegeneration;

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