Assessment of an in vitro physiological relevant model to check therapeutic strategies for glaucoma

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Abstract

Glaucoma is a chronic, progressive and heterogeneous optic neuropathy which affects in the early stage the peripheral vision and then the central vision, leading to irreversible blindness. As known, in glaucoma the trabecular meshwork represent the main tissue which is impaired by chronic oxidative stress, aging and increase of intraocular pressure. Today, the lack of human-based models, with characteristics of high repeatability and reproducibility as well, called for an high-quality in vitro model with a good degree of resemblance for the tissue or organ of interest as a basis for new drug testing. Our team has been committed to this purpose by assessment of a polyphenol mixture (PM), an active compound of a commercial eye drops for glaucoma. For this purpose, we studied the biological property of PM in counteracting chronic oxidative stress on 3D HTMCs. Preliminary qPCR analysis showed a modulation of gene levels of collagen and other ECM glycoproteins.

Introduction

Glaucoma is the second cause of blindness in the world affecting over 67 million people worldwide.1 As known, the main causes of glaucoma onset are oxidative stress and vascular alteration which impaired Trabecular meshwork activities.

The oxidative damage is an important step in pathogenesis of Primary Open Angle Glaucoma and might be a relevant target for both prevention and therapy.2 Therefore, the aim of this study was to develop an in vitro 3D human-based dynamic model of trabecular meshwork to define the key elements relating to the glaucoma onset.

Materials and Methods

3D cultures of Human Trabecular Meshwork Cells (HTMC, Cell APPLICATION INC). Were made by embedding HTMCs into 100% Corning Matrigel™ Matrix and were maintained in a millifluidic bioreactor system connected to the peristaltic pump (Live Box 1 and Live Flow, IV-Tech srl) with constant flow rate.3 To simulate chronic stress conditions 3D-HTMC cultures were exposed to H2O2 treatment (500 µM) for 2 hours followed by 22 hours of recovery, until 15 days.

Results

Confocal imaging analysis and Alamar blue assay,4 as index of proliferation/metabolic state of cultures, evidenced a good healthy state of HTMCs. Moreover, in our dynamic model an efficient response to stress was shown, since it was observed a NF-kB and TNF-α activation. To evaluate the feasibility of our dynamic HTMC 3D-model as a useful tool for evaluate therapeutic strategies for glaucoma disease, we analyzed the effects of a polyphenol mixture (PM), an active compound of a commercial eye drops for glaucoma. For this purpose, we studied the biological property of PM in counteracting chronic oxidative stress on 3D HTMCs. Preliminary qPCR analysis showed a modulation of gene levels of collagens and other ECM glycoproteins.

Taking into account these findings, our dynamic 3D-HTMC model can provide useful information on new prevention and therapeutic strategies for glaucoma.

Key words: Glaucoma; in vitro model; oxidative stress; toxicology.

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