SUPPLEMENTING PUNICALAGIN PROTECTS HUMAN RETINAL PIGMENT EPITHELIUM CELLS (ARPE-19) FROM ULTRAVIOLET RADIATION-INDUCED OXIDATIVE DAMAGE

Giuseppe Tringali[1], Beatrice Sampaolese[2], Francesca Sciandra[2], Maria Elisabetta Clementi[2]

[1]Istituto di Farmacologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome - Italy, [2]CNR-ICRM, Istituto di "Chimica del Riconoscimento Molecolare" c/o Instituto di Biochimica e Biochimica Clinica, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Università Cattolica del Sacro Cuore, Rome - Italy

Introduction: Retinal pigment epithelium (RPE) cells oxidative stress is critically implicated in the pathogenesis of age-related macular degeneration (AMD). It has long been hypothesized that exposure to solar ultraviolet (UV) radiation is cause of oxidative damage in RPE cells by producing high levels of reactive oxygen species (ROS). In fact, an imbalance between the production and neutralization of ROS by antioxidant systems is associated with chronic photo-oxidative stress. Recently studies have shown that Punicalagin, a major polyphenol abundant in pomegranate fruit, is able to protect several cell types from oxidative stress. In this framework, this study aims to establish whether Punicalagin protects RPE from ultraviolet radiation-induced oxidative damage.

Methods: We used a human cellular line of RPE (ARPE-19) treated with UV-A rays for 1, 3and 5hours hrs. This is an experimental model validated and characterized of our group previously to study the effects of radiation on RPE. In this experimental paradigm, we investigated the possible molecular mechanisms involved in the protective actions of Punicalagin. Specifically, we evaluated the cellular viability and intracellular ROS levels, as well as mRNA expression of genes involved in cellular protection by oxidative stress [Nrf2(nuclear factor erythroid 2-related factor 2), HO-1(heme-oxygenase-1) and NQ01(NAD(P)H: quinone oxidoreductase 1)]. Finally, the expression and protein levels of Bax and Bcl2, positive and negative regulators of programmed cell death, were investigated by semiquantitative PCR and utilizing a colorimetric assay kit, respectively.

Results: We found that pre-treatment with Punicalagina (24h) protected the RPE cells from exposure to UV-A radiations. In particular, pre-treatment with Punicalagina: 1) antagonized the consistent decrease in viability observed following UV-A exposure; 2) reduced the high levels of intracellular ROS associated with UV-A-induced oxidative stress; 3) reduced cellular oxidative stress, increasing the mRNA of Nrf2and its translocation at the nuclear level, as well as downstream target proteins of Nrf2, HO-1and NQO1; and 4) induced an anti-apoptotic effect by decreasing Bax and increasing Bcl2expression.

Discussion and conclusions: The results obtained show that pre-treatment with Punicalagin enhance cells survival after UV-A radiation exposure. In particular, the protective effect seem to be correlated with a control both in radical oxygen species production and in mitochondrial functions, with consequent reduction of apoptosis. In conclusion, the present findings provide preclinical evidence that Punicalagin is able to preventing UV-A-induced oxidative damage in RPE, providing a plausible therapeutic strategy for the primary prevention of early AMD.