LEUKOCYTE INTEGRIN ANTAGONISTS AS A NOVEL OPTION TO TREAT DRY AGE-RELATED MACULAR DEGENERATION

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Introduction: Age-related macular degeneration (AMD) is a complex neurodegenerative visual disorder and is the leading cause of blindness in the elderly in developed countries. Loss of vision is attributed to macular drop out of retinal pigment epithelium (RPE) cells termed "dry" AMD or to the invasion of RPE and/or retina by abnormal blood vessels, named ''wet'' AMD. Wet AMD can be treated with intravitreous application of "anti-VEGF" agents; on the contrary, there is currently no effective treatment for dry AMD, the most common form of this disease. The pathophysiology of dry AMD remains poorly understood but inflammation and immune system play a key role. RPE is a monolayer of cells situated between the neuroretina that express several adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) or vascular adhesion molecule-1 (VCAM1). Adhesion molecules are cell surface receptors that mediate adherence of leukocytes through the binding of integrins, which are α/β heterodimeric membrane receptors that mediate cell-cell and cell-extracellular matrix interactions. Targeting integrins has proven to be clinically relevant: to date, several monoclonal antibodies and small molecules directed against integrins have been approved for the treatment of multiple sclerosis, Chron's disease and thrombosis. Therefore, considering integrins as a valuable drug target, the current study was performed to elucidate the cross-talk between RPE and leukocytes investigating integrin-mediated cell adhesion and intracellular signaling. Moreover, after the characterization of RPE-leukocytes interaction, we investigated the role of small molecules, antagonists of leukocyte integrins to fight dry AMD.

Materials and methods: In vitro experiments were performed in suitable cell lines: ARPE-19 (a human retinal pigment epithelial cell line), Jurkat (human T lymphocytes) and THP-1 cells (human monocytic cell line). ARPE-19 and immune cells were co-cultured for different time points (0-1-16-24-48h); adhesion molecule expression and apoptosis were evaluated by flow cytometry and intracellular signaling activation by western blotting. Furthermore, $\alpha 4\beta 1$ and $\alpha L\beta 2$ integrin antagonists (1-100nM; 1-16-24-48h), synthesized in the Department of Chemistry (University of Bologna), were used to ascertain if these small molecules may influence the cross-talk between immune cell and ARPE-19 cells.

Results: When ARPE-19 cells were co-cultured with immune cells, we observed a significant reduction of ICAM-1 expression on ARPE-19 cells whereas integrin-mediated intracellular signaling was activated. In addition, immune cells induced an increase in ARPE-19 cells apoptosis after 16 and 24h of co-culture. Moreover, immune cells-ARPE-19 interaction activates integrin-mediated intracellular signaling. Integrin antagonists proved to be effective in inhibiting RPE cell death and antagonizing the interaction between immune cells and RPE.

Discussion and conclusion: On the basis of these preliminary results, antagonists for $\alpha 4\beta 1$ and $\alpha L\beta 2$ integrins may represent novel, valuable tools for the development of new therapeutic agents useful to fight dry AMD. Nevertheless, further studies are needed to better characterize the effects of integrin antagonists on RPE-immune cells cross-talk.