In vitro effects of mediators derived from porcine vascular stem cells on intestinal expression of cannabinoid and opioid receptors under inflammatory conditions.

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Background. Inflammatory Bowel Diseases (IBDs) are chronic intestinal disorders characterized by recurrent inflammatory episodes associated with an aberrant immune response to intestinal lumen antigens. The opioid and cannabinoid systems participate to various bowel functions and their expression is altered in patients with IBDs (Philippe et al., 2006; Storr et al., 2009). Mesenchymal stromal cells (MSCs) are multipotent cell types located in almost every tissue undergoing self-renewing processes. Upon inflammatory stimuli, MSCs exert an immunomodulatory action through cytokine release (Krampera et al., 2006) and are viewed as a potential therapeutic strategy for inflammation-driven disorders, including IBDs (Dave et al., 2017). Recently, precursor cells derived from porcine aortic vascular tissue (pAVPCs) were phenotypically and functionally characterized as MSC-like cells (Zaniboni et al., 2015).

Aim. The present project aims at evaluating the effect of pAVPCs mediators on the expression of μ opioid receptor (MOR) and cannabinoid receptor 2 (CB2) under inflammatory conditions.

Methods. To this purpose primary cultures of enteric ganglia obtained from guinea pig myenteric plexus were exposed to ascending concentrations of bacterial lipolysaccharide (LPS, 0.1-1-10g•L-1) and/or pAVPCs supernatants for 24h, after 2 or 6 days of cultures. Cells were then fixed in paraformaldehyde and processed for double immunofluorescence for MOR and CB2 localization on GFAP positive glial cells and HuB/D positive neurons. Neuronal and intestinal epithelial cell lines (SH SY5Y and CaCo2, respectively) were treated with LPS 0.1-1-10g•L-1 and/or pAVPCs conditioned medium (CM) for 6-18-24 h. Quantification of MOR in SH SY5Y and CB2 in CaCo2 protein extracts was performed by Western Blot analysis. Results. Immunofluorescence cell counts showed a higher number of GFAP-immunoreactive glial cells in enteric ganglia treated with LPS 1g•L-1 (+10% $p<.05$) and an increased number of CB2 positive cells. This change was not observed in the co-treatments with LPS 1g•L-1 and CM. Conversely, a lower number of MOR positive glial cells was observed in the LPS 0,1-1g•L-1 treated groups and a number of MOR positive cells was counted in LPS 1g•L-1 and CM co-treatment. The observed variations of cell number expressing the targets under study were observed predominantly in glial cells and in the experiments performed after 2 days rather than 6 days of culture. Preliminary data show a 5-fold increase of MOR expression in SH SY5Y treated with LPS 0,1g•L-1 for 24h, whereas no statistically significant variation in cell number was observed in the samples co-treated with LPS 0,1g•L-1 and CM. Similarly, CB2 protein expression was enhanced in CaCo2 cells treated with LPS 0,1g•L-1 , this enhancement was not observed in the co-treated samples.

Conclusions. Taken together, these results suggest an inhibitory effect of pAVPCs mediators on LPS-induced alteration of CB2 and MOR expression and warrant further research into their therapeutic potential.

References

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