mechanism is still obscured. During the last years, extracellular vesicles (EVs) of endosomal origin with the physical characteristics of exosomes have been emerged as organelles performing intercellular communication. EVs/exosomes may alter the immune status or even the physiological function of recipient target cells through shuttling of their cargo molecules.
Methods: In this work we have characterized EVs/exosomes isolated from BAL fluid of patients with and without ALI/ARDS, using physical, morphological and biochemical approaches. Furthermore, we provide biochemical and morphological evidence for the presence of an EV pool of sPLA2-IIA in the BAL fluid of ARDS patients.
Results: Exosomal type extracellular vesicles were isolated from BAL fluid of patients with and without ARDS and characterized on the basis of their density, diameter, the presence of tetraspanins CD63 and CD81 and the absence of GRP78. In the EVs of exosomal type from ARDS patients we identified secretory phospholipase A2 type II (sPLA2-IIA) and in sporadic samples pcPLA2. by immunofluorescence and immunogold TEM.
Summary/Conclusion: To our knowledge, this is the first description of exosomal localization of a secreted PLA2 isoform in human samples. Exosomal sPLA2-IIA might be involved in the responsiveness of recipient cells in the lung during the development of ARDS, in a functionally distinct manner from soluble sPLA2 present in BAL fluid, which is presumably implicated in lung surfactant hydrolysis during the course of the disease. The presence of PLA2 isoenzymes on EVs may reveal new insight into the development and propagation of lung inflammation, but can also help adopt appropriate management approaches and thus, new ways for patients' treatment.

PT09.11

Different anti-inflammatory effects of extracellular vesicles from adipose-derived mesenchymal stem cell or keratinocyte cell line on osteoarthritic cartilage

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Background: Osteoarthritis (OA) is a joint condition associated with articular cartilage loss, low-grade synovitis and alterations in subchondral bone and periarticular tissues. In OA, the interest for mesenchymal stem cell (MSC)-EV therapeutic applications has increased. However, there is an increasing concern about the reproducibility of recent EV publications.
We have assessed the immunomodulary properties of adipose-derived MSCs (AD-MSCs) microvesicles (MV) and exosomes (EX) in OA chondrocytes and compared their effects with EVs from a different biological source.
Methods: AD-MSCs from abdominoplasty fat and immortalized keratinocytes (HaCaT) were cultured with appropriate media supplemented with EV free human serum. EVs were isolated from conditioned media by differential centrifugation and characterized by resistive pulse sensing.
Cartilage explants and primary chondrocytes were obtained from knee specimens of advanced OA. Both were stimulated with interleukin (IL)-1β (10 ng/mL) and treated with MSC- or HaCaT-MV (3.6×10^7 particles (p)/mL) or EX (7.2×10^7 p/mL) for 24 h. Then, levels of IL-6, IL-10 and TNFα were measured.
Results: RPS revealed distinct size and concentration EV signatures from AD-MSCs (MV: 317 ± 54 nm and 8×10^9 p/mL; EX: 151 ± 27 nm and 4×10^{10} p/mL) or HaCaT (MV: 281 ± 2 nm and 7×10^{10} p/mL; EX: 105 ± 1 nm and 1.1×10^{12} p/mL).
MSC-EV treatment of OA cartilage explants and chondrocyte cultures reduced the inflammatory cytokines IL-6 and TNFα with respect to those solely stimulated IL-1β. The anti-inflammatory cytokine IL-10 increased with respect to explants or cells solely stimulated with IL-1β. On the contrary, the levels of the same cytokines were not affected by treatment with HaCaT-EVs.
Summary/Conclusion: Administration of EV may have potential pharmacological applications in OA. However, experimental procedures to avoid data artefacts are currently lacking; in this regard, the use of non-related EVs as negative controls has proven useful. Interestingly, cell line HaCaT EVs had less deviation in size, and were obtained in higher concentrations, compared to EVs from primary cell cultures. Further studies on EV properties may lead to new and more specific therapeutic targets based on the interaction between AD-MSC-EVs and cells.
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PT09.12

Tiotropium inhibits the release of pro-inflammatory extracellular vesicles by acetylcholine-stimulated lung epithelial cells

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Background: Tiotropium is a long-acting muscarinic antagonist routinely used as a bronchodilator in chronic obstructive pulmonary disease (COPD). Based on its role in preventing acute exacerbations of COPD, it has been speculated that besides its known bronchodilator properties tiotropium also exerts anti-inflammatory effects. We have shown that extracellular vesicles (EV) generated by mononuclear cells induce a pro-inflammatory phenotype in human lung epithelial cells. The aim of this study was to investigate whether muscarinic stimulation induces the generation of pro-inflammatory EV by alveolar (A549) and bronchial (16HBE) epithelial cells and whether tiotropium modulates such effect.
Methods: The generation of A549- and 16HBE-derived EV induced by acetylcholine (Ach; 1 mM; 1 h) in the presence or in the absence of tiotropium was investigated through a prothrombinase assay. Ach-induced A549-EV and 16HBE-EV were incubated overnight with A549 and 16HBE cells, respectively, and the concentrations of IL-8 and MCP-1 in the conditioned medium assessed by ELISA.
Results: Ach stimulation of A549 cells caused an increase in EV from 0.225±0.088 to 0.381±0.087 mM PS ($p < 0.05$; paired t-test). EV generated by Ach-stimulated A549 cells caused an autocrine stimulation of the synthesis of IL-8 (487±242 pg/mL vs. 1896±211 pg/mL for unstimulated and EV-stimulated A549 cells, respectively) and MCP-1 (1299±237 pg/mL vs. 5973±924 pg/mL for unstimulated and EV-stimulated A549 cells); $p < 0.05$ for both comparisons; paired t-test. Preincubation of cells with tiotropium prior to Ach stimulation caused a dose-dependent inhibition of EV generation that reached maximum at 50 pg/mL (0.225 ±0.101 nM PS). Similar results were obtained with 16HBE cells.
Summary/Conclusion: Muscarinic stimulation causes the generation of pro-inflammatory EV by human lung epithelial cells that is inhibited by tiotropium. This observation could contribute to explain the effect of tiotropium in the reduction of acute exacerbations of COPD.

PT09.13

Endothelial Progenitor Cell Exosomes Improve the Outcome of a Murine Model of Sepsis

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Background: Microvascular dysfunction leads to multi-organ failure and mortality in sepsis. Our previous studies demonstrated that administration of exogenous endothelial progenitor cells (EPCs) confers protection in sepsis as evidenced by reduced vascular leakage, improved organ function and increased survival. We hypothesized that EPC-exosomes protect the microvasculature through the transfer of miRNAs.
Methods: Mice were rendered septic by cecal ligation and puncture (CLP), and EPC-exosomes were administered intravenously at 4 h