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## 802.CHEMICAL BIOLOGY AND EXPERIMENTAL THERAPEUTICS

### Study of the Effect of CD34+ Umbilical Cord Blood Stem Hematopoietic Cell Microparticles on the Induction of Oxidative Stress in HL-60 Promyelocytic Cells and in Umbilical Cord Blood Mononuclear Cells

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*Objective* Oxidative stress is defined as a disturbance between prooxidant-antioxidant balance and its main cause are molecules or ions such as reactive oxygen species. Antioxidant enzymes have a primary role against oxidative stress, while its effects can be tracked from the oxidation of biological molecules such as fatty acids. Microparticles are small membrane vesicles released from different cell types upon activation or during apoptosis such as platelets, erythrocytes, leucocytes and endothelial cells. Microparticles consist of cytosolic molecules and membrane protein antigens. They can interact with other cells activating multitude functions that are associated with many pathological conditions.
The present study aims to investigate a possible correlation between oxidative stress and hematopoietic stem cell derived microparticles in HL-60 and mononuclear cells (MNCs).
*Methodology* The source of MNCs as well as CD34 $^{+}$ microparticles (CD34+MPs) is the umbilical cord blood (UCB). The units (n=17) that were used in this study, were rejected as not appropriate for processing due to low volume. The isolation of the mononuclear cells is accomplished after density gradient centrifugation on lymphoprep. CD34+MPs were isolated from the plasma of UCB after centrifugation and were purified using magnetic bead MACS (Miltenyi Biotec). The number of CD34+ MPs was estimated by flow cytometry using CD34-PE and Annexin V-FITC Abs. Cells are then co-incubated with the CD34+MPs for 24 and 48 hours. Finally, the activity of the antioxidant enzymes superoxide dismutase (SOD), glutathione reductase (GR) and glutathione S-transferase (GST) as well as the concentration of malondialdehyde (MDA) produced by lipid peroxidation are measured in the samples using spectrophotometry.
*Results* The results from the measurements that were conducted, displayed a statistically significant increase in the antioxidant activity of the samples in the presence of CD34+MPs in both HL60 and MNCs cells after 24 and 48 hours of incubation. Specifically SOD activity was significantly increase in CD34+MPs compared to the control in HL60 after 24 hrs incubation (4.38x10 $^{-1}$ *vs.*2.24x10 $^{-1}$units/mg) and in MNCs (5.51x10 $^{-1}$ *vs.* 3.52x10 $^{-1}$units/mg) The GR activity was increased significantly in HL60 treated with CD34+MPs compared to the control (4.96x10 $^{-2}$ *vs.*3.09x10 $^{-2}$units/mg) as well as in MNCs (5.25x10 $^{-2}$ *vs.*3.23x10 $^{-2}$ units/mg). Similarly the GST ativity increased in the presence of CD34+MPs compared to the control (6.3x10 $^{-3}$ *vs.* 2.96x10 $^{-3}$units/mg) in HL60 and in MNCs (5.34x10 $^{-3}$ *vs.*3.28x10 $^{-3}$units/mg).The MDA concentration produced by lipid peroxidation was increased in HL60 after CD34+MPs treatment compared to the control (5.59 *vs.*2.84$\mu$M after 24 hrs and 10.08 *vs.*3.43 after 48hrs) and in MNCs (6.34 *vs.*3.04 after 24 hrs and 8.76 *vs.*3.52$\mu$M after 48 hrs incubation).
*Conclusions* In this study we have shown the induction of oxidative stress in the leukemic cell line HL60 and in the mononuclear cells from cord blood by CD34 $^{+}$MPs. Therefore the CD34+ microparticles can promote oxidative stress resulting in the activation of inflammation or apoptosis.

**Disclosures** No relevant conflicts of interest to declare.

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