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ISOLATION AND EXVIVO EXPANSION OF HUMAN UMBILICAL CORD BLOODDERIVED CD34+ STEM CELLS AND THEIR COTRANSPLANTATION WITH OR WITHOUT MESENCHYMAL STEM CELLS

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Introduction: Umbilical cord blood (UCB) contains a high number of primitive progenitor cells. This characteristic allows UCB to be as a source of hematopoietic progenitors for clinical transplantation. However the rate of UCB CD34+ stem cells graft is low. Mesenchymal stem cells (MSC) have been implicated in playing an important role in hematopoietic stem cell engraftment.

Aim: So that in this study we examined the effect of human MSC on engraftment of human umbilical cord blood (UCB)-derived CD34+ cells in irradiated Balb/C mice.

Methods: Human UCB CD34+ cells were obtained from full-term normal deliveries by using an immunomagnetic separation technique and MSC were isolated by standard methodology from human bone marrow. The direct determination of the absolute count of CD34+ was assayed by Flow cytometry. Isolated MSC characterized according by flow cytometric determination of cell-surface antigen CD166 and CD105 and their morphology. Viability test was performed by trypan blue staining. Isolated CD34+ cells were cultured in Stemline Hematopoietic stem cell expansion medium supplemented with 100ng/ml SCF, and 100ng/ml TPO in 24-well plates and incubated at 37°C in a fully humidified atmosphere with 5% CO2, and maintained over three weeks and half the medium was exchanged twice a week. MSC were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS). Irradiated (7 Gy) Balb/C mice were transplanted intravenously with 0.2 to 1.0 ^{x} 106 human UCB CD34+ cells in the presence or absence of 0.25 to 1 ^{x} 106 human bone marrow-derived MSC. The mice in every group on day 11 after transplantation were killed and their spleen dissected. In every group colony assay were performed. For better study of colony in the spleen H&E staining was performed. For approving the presence of human stem cells in colony, UCB CD34+ cells labeled with super paramagnetic iron oxide (SPIO) were transplanted. After establishing the presence of colonies in spleen, Prussian blue staining was performed.

Result: The average number of CD34+ were obtained from every sample was 25 to 50^{x}104 and MSC was 7^{x}104 per samples. Flow cytometry assay showed that up to 90% purity of CD34+ cells and 96% for MSC and trypan blue staining showed that percentage of viable cell was 100%. Also after three weeks the cell numbers were reach 1000-fold increasing of CD34+ and after two month 500-fold increasing of MSC. Cotransplantation of low doses of UCB CD34+ cells (0.2 and 0.3 ^{x} 106) and MSC (0.5 and 1 ^{x} 106) resulted in a significantly increasing in colony forming unit spleen, in comparison with engraftment of UCB CD34+ stem cells without MSC after 11 days (P<0.01). After Prussian blue staining Fe+2 granules was observed. This indicated these cells in the colony were UCB CD34+ stem cells that were engrafted.

Conclusion: In conclusion the results showed that usage two cytokines (SCF, TPO) was adequacy for expansion of UCB CD34+ cells. Also cotransplantation of MSC with UCB CD34+ cells; promote engraftment of UCB CD34+ cells.

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