Local Transplantation of Granulocyte Colony Stimulating Factor-Mobilized Human Peripheral Blood Mono Nuclear Cells for Unhealing Bone Fracture

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INTRODUCTION

We previously reported that transplantation of human peripheral blood (hPB) CD34+ cells, an endothelial/hematopoietic progenitor cell-enriched cell population (1), contributes to fracture healing via vasculogenesis and osteogenesis in an immunodeficient rat model of unhealing fracture (2-5). On the other hand, in the field of revascularization, not only hPB CD34+ cells but also hPB total mononuclear cell (MNC) transplantation for hind-limb ischemia or myocardial ischemia have shown their therapeutic efficiency to enhance ischemic neovascularization (6, 7). In the clinical setting, the use of hPB MNCs is more reasonable than hPB CD34+ cells from economic and technical standpoints, but there were some reports showing a higher therapeutic potential in hPB CD34+ cells than hPB total MNCs. Based on the controversy, we first performed experiments to prove a hypothesis that hPB MNC transplantation may also contribute to fracture healing via vasculogenesis/angiogenesis and osteogenesis. To this end, comparison of the findings in the current study to our previous studies allowed us to discuss which cells are more suitable for a cell therapy of fracture healing.

MATERIALS AND METHODS

Cell preparation: We prepared for Granulocyte Colony Stimulating Factor (G-CSF) mobilized hPB MNCs of healthy male (Lonza), and characterized them by Fluorescence-Activated Cell Sorting (FACS) analysis to measure a content of CD34+ cells.

Animal Model: A reproducible model of femoral fracture was created in nude rats with the periosteum cauterized, which lead to the nonunion 8 weeks post-fracture (8).

Cell Transplantation: The rats received local administration of following materials with atelocollagen after fracture creation; high dose hPB MNCs (Hi, 107) containing 105 CD34+ cells, low dose hPB MNCs (Lo, 105) containing 105 CD34+ cells or PBS.

RESULTS AND DISCUSSION

Characterization of hPB MNCs: hPB MNC fraction had about 1% of CD34+ cells as determined by FACS analysis (Figure 1).

Human cell-derived vasculogenesis and osteogenesis: Immunohistochemical staining (IHC) and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) for human-specific endothelial cell (EC) or osteoblast (OB) markers was performed using tissue samples harvested at week 2. Differentiated human ECs and OBs were identified as UEA-1 lectin-positive cells and hOsteocalcin (OC)-positive cells in animals receiving Hi dose hPB-MNCs, but not in other groups. RT-PCR analysis of tissue RNA isolated from the peri-fracture site demonstrated that the gene expression of each human-specific EC (VE-cadherin, CD31) and OB (OC, Collagen 1A1)-related marker in animals receiving Hi dose hPB MNCs, but not in other groups.

CONCLUSION

Local transplantation of hPB MNCs contributes to fracture healing via vasculogenesis/angiogenesis and osteogenesis. However, when we compare the efficacy of 10^7 MNCs to that of 10^5 CD34+ cells to adjust the number of CD34+ cells, their potential for fracture healing is inferior to CD34+ cells.

REFERENCES