Local Transplantation of Ex-vivo Expanded Bone Marrow-derived CD34 Positive Progenitor Cells Accelerate non-Union Fracture Healing

INTRODUCTION

Failures in fracture healing are mainly caused by a lack of neovascularization. We have previously demonstrated that local transplantation of G-CSF-mobilized peripheral blood (GM-PB) CD34+ cells, an endothelial/hematopoietic progenitor enriched cell population, contributed to fracture healing via vasculosugeness and osteogenesis. However, the scarcity of CD34+ cells in PB and the biological side effect of G-CSF administration and apheresis still remain to be solved problems in clinical settings. In terms of expanding clinical application, we postulated the hypothesis that local transplantation of same number of not only bone marrow (BM) CD34+ cells but also culture expanded BM-CD34+ cells as that of GM-PB CD34+ cells might exhibit similar or more potent therapeutic potential for fracture healing without increasing the original number of cells for transplantation. In this study, we performed a series of experiments to compare the therapeutic effects of local transplantation of expanded BM CD34+ cells, BM CD34+ cells and GM-PB CD34+ cells using a rat unhealing fracture model.

MATERIALS AND METHODS

In-vitro Study: To assess the ability of angiogenesis and osteogenesis, we performed colony forming assay, tube formation assay and osteoinduction assay with the cEx derived CD34+ cell transplantation leads to functional bone healing in fracture. Biomechanical evaluation by a three-point bending test was performed at week 8 in all groups. cEx-BM CD34+ group and GM-PB CD34+ group showed significantly high values of stress test ratio. Enhancement of intrinsic vascularization and osteogenesis; Vascularity in peri-fracture sites was assessed by immunohistochemical staining with CD31 and isletin B4 at week 2. Capillary density was significantly great in cEx-BM CD34 group compared with the other groups. Osteoblast staining with rat OC at week 2 also revealed that Osteoblast density was great in both cEx-BM CD34 group and BM CD34 group compared with the other groups.

RESULTS AND DISCUSSION

Characterization of Expanded Cells: Total cell number of BM CD34+ cells was increased 227-276.6 times after culture expansion for one week. FACS analysis demonstrated 99.3% and 69.9% positivity of CD34 expression in BM CD34+ cells and cEx-BM CD34+ cells, respectively. cEx-BM CD34+ cells were positive for CD31, CD44, CD133, CD90, CD105, and CD166, and negative for c-kit and STR1-BM derived CD34+ cells showed high capacity of colony/tube formation and osteogenic differentiation in vitro: After 16 days in culture with methylcellulose- or Matrigel-based medium, number of small and large colonies got was significantly great, and formed tubes were also frequently observed in cEx-BM CD34 group compared with the other groups. When cells were cultured in osteogenic differentiation medium for 3 weeks, calcium deposit assessed by Alizarin red staining was significantly greater in cEx-BM CD34 group and BM CD34 group but not in GM-PB CD34 group by osteogenic induction. These data suggest that BM derived CD34+ cells have high differentiation potential into osteoblasts.

Human cell-derived vasculosugeness and osteogenesis: Differentiated human endothelial cell and osteoblast were identified as UEA-1 lectin-positive cells and human osteocalcin (hOC) positive cells in animals receiving cEx-BMCD34+ cells and CD34+ cells, but not in PBS groups by the immunostaining and RT-PCR. cEx-BM CD34+ cells exhibit potent therapeutic potential in fracture healing: Fracture healing was assessed radiographically 8 weeks after surgery. Fractures healed with bridging callus formation in 100% animals of cEx-BM CD34 group, 80% animals of BM CD34 group and 50% animals of GM-PB CD34 group, while fracture sites in all animals receiving PBS showed no bridging callus formation and finally fell into non-unions. In micro-computed tomography (μCT) analysis, both callus volume and bone density at fracture site were significantly greater in cEx-BMCD34 group than the other groups. (Fig.1) In histological evaluation, cEx-BM CD34 group showed the best fracture healing score assessed by Allen’s classification among all groups.

DISCUSSION AND CONCLUSION

Our 7-day culture expansion technique allowed us to obtain around 23 times of BM CD34+ cells maintaining 60% purity of CD34 positivity. Moreover, the culture expanded BM CD34+ cells exhibited striking therapeutic efficacy for unhealing fracture promoting neovascularization and osteogenesis in sites of fracture even in the same number of freshly isolated BM CD34+ cells or GM-PB CD34+ cells.

REFERENCES