Local Delivery of Granulocyte Colony Stimulating Factor-Mobilized CD34-Positive Progenitor Cells Using Bioscaffold for Therapeutic Modality of Unhealing Bone Fracture

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Introduction: Failures in fracture healing are mainly caused by a lack of vascularization. We previously reported that systemic administration of adult human circulating CD34+ cells, an endothelial/hematopoietic progenitor cell (EPC/HPC)-enriched cell population (1), contributes to functional fracture healing via vasculogenesis and osteogenesis in an immunodeficient rat model of non-healing fracture (2). In addition, we clarified that fracture induces mobilization of EPCs from bone marrow (BM) to circulation and recruitment of the mobilized EPCs into fracture sites, thereby augment neovascularization during the process of bone healing (3). Based on these scientific backgrounds, to widen clinical application, we performed experiments to prove a reasonable hypothesis that human peripheral blood CD34+ cells may have potential of differentiating into osteocytes in vitro, and that local transplantation of granulocyte colony-stimulating factor mobilized peripheral blood (GM-PB) CD34+ cells may further contribute to fracture healing.

Materials and Methods: Osteogenic differentiation in vitro We prepared for the supernatants of BM mesenchymal stem cell-cultured medium as conditioned medium (CM). We seeded GM-PB CD34+ cells in standard medium with 10% CM, then the cells were cultured in osteogenic medium.

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 (4).

Cell Transplantation The rats received local administration of following materials with atelocollagen after the fracture creation; high (Hi, 10³), middle (Mid, 10²) or low dose (Lo, 10¹) GM-PB CD34+ cells or PBS alone.

Results: Osteogenic differentiation in vitro Treatment of the first passage cells in osteogenic condition resulted in a morphological transformation to cuboidal shape, and matrix mineralization was demonstrated by alizarin red staining. The mRNA of osteocalcin and collagenX1A1 was expressed in osteogenesis-induced cells.

Therapeutic superiority of local transplantation To prove therapeutic superiority of local transplantation over systemic infusion, rats received 10⁵ CD34+ cells locally for Mid vs Lo or PBS group). As one of the mechanisms underlying enhancement of angiogenesis and osteogenesis following CD34+ cell therapy, upregulation of angiogenesis and osteogenesis-related cytokines at the peri-fracture site could be considered. We have performed real time RT-PCR to quantify the expression of rat vascular endothelial growth factor (rVEGF) and bone morphogenetic protein 2 (rBMP-2) around the fracture sites. Expression ratio of rVEGF to rGAPDH at week 2 was greater in Hi group compared with other groups, as well as in Mid group than Lo and PBS groups (Hi, 1.13±0.284; Mid, 1.08±0.269; Lo, 1.03±0.215; PBS, 1.03±0.231 respectively. P<0.01 for Hi vs Lo or PBS group, P<0.05 for Hi vs Mid group and for Mid vs Lo or PBS group). The expression ratio of rBMP-2 to rGAPDH at week 2 was also significantly greater in Hi group compared with other groups, as well as in Mid group than Lo and PBS groups (Hi, 1.028±0.276; Mid, 0.991±0.271; Lo, 0.92±0.216; PBS, 0.907±0.244 respectively. P<0.01 for Hi vs Lo or PBS and for Mid vs PBS group, P<0.05 for Hi vs Mid group and for Mid vs Lo group).

Serial improvement of blood flow at fracture site Laser Doppler perfusion imaging was performed at week 0, 1 and 2. There was no significant difference in the blood flow ratio (BFR) (fractured site/ contralateral site) 1 hour after fracture creation among each group, while BFR at week 1 was significantly higher in Hi group compared with the other groups, as well as in Mid group than Lo and PBS groups. At week 2 BFR was also significantly higher in Hi group compared with Lo and PBS groups, as well as in Mid group than PBS group.

Radiographic and histological evidence of fracture healing In all animals receiving Hi dose and 50 % of animals receiving Mid dose CD34+ cells, fracture radiographically healed with bridging callus formation, while fracture site in all animals receiving Lo dose cells or PBS showed no bridging callus formation and fell into non-unions. In histological evaluation, the degree of fracture healing assessed by Allen’s classification (5) were significantly greater in Hi group than the other groups at week 8, as well as in Mid group than Lo and PBS groups (Hi, 3.8±0.13; Mid, 2.1±0.16; Lo, 0.4±0.48; PBS, 0.0±0.00, respectively. P<0.01 for Hi vs Lo or PBS group and Mid vs PBS group, P<0.05 for Hi vs Lo or PBS and Mid vs PBS group, P<0.05 for Hi vs Mid group and Mid vs Lo group).

Discussion: These results strongly suggest that local transplantation of GM-PB CD34+ cells with atelocollagen scaffold is practically potent strategy for therapeutic vasculo-osteogenesis in fracture healing.