Improvement of Osteonecrosis by Administration of Human Peripheral Blood Derived CD34-positive Cells in Rat Osteonecrosis Model

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Introduction: An impeded blood flow through the femoral head is incriminated in the etiopathogenesis of osteonecrosis of the femoral head. Treatment of osteonecrosis of the femoral head still presents a clinical challenge. Recent clinical study demonstrated that transplantation of bone-marrow cells, as a source of mesenchymal stem/progenitor cell fraction, could be a promising new approach for osteonecrosis of hip. On the other hand, it was revealed that the human peripheral blood derived CD34-positive cells, known as an endothelial progenitor cell fraction, have a potential of vasculogenesis and osteogenesis in the rat nonhealing fracture model. Inspired these data, we speculated that transplantation of the CD34-positive cells could be a more powerful cell-based therapy for the osteonecrosis of femoral head through the vasculogenesis and osteogenesis. The purpose of this study is to prove the therapeutic potential of the human peripheral blood derived CD34-positive cells for osteonecrosis using rat femoral head necrosis model and to develop new cell-based therapy in clinical settings.

Materials and Methods: The institutional animal care and use committees of the university of the Hiroshima approved all animal procedures, including human cell transplantation. G-CSF mobilized CD34-positive cells were used in this study. At first, we developed vascular deprivation-induced rat femoral head necrosis models modified previous report using 8-week-old female athymic nude rats. The cervical periosteum of the femur was coagulated by heat after dislocation of the head for vascular deprivation. After production of osteonecrosis, rats received an intravenous transplantation of 1x10⁵ CD34-positive cells with 100μl of PBS or the same volume of PBS without cells (n=20 in each group). We evaluated 2 and 4 weeks after transplantation. First, micro-CT imaging was performed to assess the structure of femoral head and neck in vivo. Next, gross findings of the femoral head, neck, and articular surface was evaluated macroscopically. Neovascularization was evaluated macroscopically by angiography with colored compound. For histological evaluation of vasculogenesis and osteogenesis induced by transplanted CD34-positive cells were evaluated by immunofluorescent staining with antibodies for von Willebrand factor, osteocalcin, and human nuclear antigen.

Results: In the micro-CT imaging, all cases were recognized the neck fracture in the control group. On the other hand, no fracture was confirmed in the CD34 group at each time point (Image 1), although thinning of articular cartilage was observed. Neovascular formations visualized with angiography were observed around the femoral neck and the surface of femoral head only in the CD34 group (Image 3). Immunofluorescent staining demonstrated human CD34-positive cell-derived vasculogenesis and osteogenesis as previously reported. Differentiated human endothelial cells and osteoblasts were identified in the improved femoral head immunohistologically.

Discussion: The results of our study demonstrated that transplanted human cells could have therapeutic potential for osteonecrosis in athymic nude rat models. As previous report, we showed that transplanted cells were able to contribute to vasculogenesis and osteogenesis with differentiation themselves into endothelial cells and osteoblasts. In clinical situations, bone-marrow cell transplantation has been already applied to osteonecrosis of the femoral head. However, circulating CD34-positive cells, which have no limitation in ethical and technical problems, could be isolated from peripheral blood easily. Moreover, this purified cell fraction, considered as hematopoietic/endothelial progenitors, might be a more powerful cell source for organogenesis than bone-marrow cells consisted of heterogenous populations. In conclusion, this new therapeutic approach using human purified cell fraction could be a future ideal treatment of early-stage osteonecrosis of the femoral head in clinical settings.


In the micro-CT images 4 weeks after transplantation. Femoral neck fracture was observed only in the control group.

Neovascularization was clearly demonstrated at femoral neck and head only in the CD34 group.