Reparative Reaction after Ischemic Event in Human Femoral Neck Fracture

Introduction: Osteogenesis and angiogenesis are closely associated with the reparative process in damaged bone. When reparative reaction in human osteonecrosis (ON) is investigated using femoral head samples, we cannot detect early phase of the reparative reaction, because samples are harvested in total hip arthroplasty, almost all samples show progressed stages, and a long period has passed since ON occurred. Concerning animal models, it is always doubtful whether animal ON is the same as human ON. Therefore, in order to elucidate reparative reaction of human bone tissue after ischemic event, 58 human femoral head samples in femoral neck fracture (PFN) were investigated.

Methods: There were 9 males and 49 females with a mean age of 80 years (range, 58-97 years). There were 10 hips for Garden stage 3 and 48 hips for Garden stage 4. The mean duration from onset to the surgery was 6 days (range: 1 to 14 days). The 58 whole femoral heads were fixed in formalin and decalcified using EDTA. Thereafter, the femoral heads were cut on the coronal plane, embedded in paraffin, and cut into 5 μm sections. HE staining, TRAP staining, and immunohistochemical staining were performed. The following antibodies were also used in whole femoral heads at the specified dilutions: a rabbit monoclonal IgG against HIF-1α (1:100), a rabbit monoclonal IgG against an N-terminal epitope of human VEGF (1:100), and a rabbit polyclonal IgG against FGF-2 (1:100). Semiquantitative estimation of TRAP positive cells and immunoreactivity was performed using the ×100 magnified histology according to Sternberger and Radke, in the following three area, which included the ligamentum teres insertion area, the retinaculum area, and the fracture site. Four grades of staining were defined: negative (G0), weak (G1), medium (G2), and strong (G3). We then investigated the relationships between these semiquantitative results and the clinical characteristics including Garden stage and the preoperative duration after fracture. As control, one femoral head in a patient who underwent wide resection for metastatic acetalubar tumor was used.

Results: TRAP-positive cells were detected in 8 hips (14%) (Table1). They existed around the fracture site in 8 hips, while around the teres insertion and retinaculum in 7 hips (Figure 1). In control, they were detected slightly around the teres insertion and retinaculum (G1). Around the fracture site, HIF-1α expression was detected in 17 hips (29%) mainly at the endothelial cells (Figure 2), VEGF was expressed at the edematous area in 42 hips (72%) (Figure 3), while FGF-2 was detected widely in the marrow cells in 41 hips (71%) (Figure 4) (Table1). Although there were no significant differences in TRAP staining or in HIF-1α expression between Garden stage 3 and stage 4 hips, there were significant differences in HIF-1α and FGF-2 expression concerning the preoperative duration after fracture between ≤3 days hips and >3 days hips.

Discussion: TRAP-positive cells were detected around the teres insertion and retinaculum in PFN and the control hips, as the same findings in early stage of the femoral head osteonecrosis. TRAP-positive cells were recruited through the teres insertion and the retinaculum around the fracture site. HIF-1α and FGF-2 expression was detected more frequently in ≤3 days hips than >3 days hips. These findings are representative for early expression of HIF-1α followed by VEGF and FGF-2 expression under hypoxic conditions after ischemic event in human bone.

These findings also represented the reparative reaction process after fracture in human bone. As the systemic reaction, serum concentrations of VEGF and FGF-2 in patients with long bone fractures have been reported to increase within the first two weeks after the fracture. The local expression of VEGF and FGF-2 in the present study may support increased serum concentration of VEGF and FGF-2 as systemic reaction.