Angiogenesis is implicated as a cause of many diseases as well as an inevitable repair process. In osteoarthritis (OA) knees, pathological changes in the subchondral bone are considered to be related to disease initiation, progression, and a potent source of knee pain. Previous studies indicated that angiogenesis at the osteochondral junction might facilitate the progression of OA. Despite the presence of vascular invasion into the cartilage from the subchondral bone in end-stage OA knees, it is largely unknown when vascular invasion occurs. Since vascular invasion depends on the angiogenic activities of tissue, we hypothesized that there might be a specific period with elevated angiogenic activities during the development of OA.

The purpose of this study was to investigate the change of angiogenic activities during the development of OA by biochemically and histochemically in rabbit OA model.

Methods: OA was surgically induced by anterior cruciate ligament transection (ACLT) in left knee of 12 months old female New Zealand white rabbits. Contra lateral knees were sham operated. Animals were necropsied at 2, 4, 6, 8, and 12 weeks postsurgery. Six animals were allocated each time point. All the knees were examined macroscopically and three rabbits for histological evaluation and three were for angiogenic activity analysis. Histologic evaluation was performed with hematoxylin and eosin, safranin-O staining. OA changes were evaluated by the grading score of OARSI.

For angiogenic activity analysis subchondral bone and cartilage of the medial femoral condyle (MFC) and those of lateral femoral condyle (LFC) were obtained from each knee at each time point. The specimens were co-cultured with human umbilical vein endothelial cell (HUVEC) and fibroblasts using Angiogenesis Kit (KURABO, Osaka, Japan) for 11 days according to the manufacturer’s instruction. After formation of the vessel lumens were identified, the vessels were immunostained with anti-CD31 antibodies and four parameters concerning angiogenic activities, i.e. the length, area, number of joints and paths of newly created vessels, were analyzed automatically by Angiogenesis Image Analyzer (KURABO, Osaka, Japan). To correct age and tissue specific angiogenic activities, each parameter of OA knees was divided by corresponding sham operated knees.

Results: The characteristic OA features were detected from 4 weeks in MFC, and from 6 weeks in LFC macroscopically and microscopically and the grading score of OARSI increased time dependently. The calculated parameters of the angiogenesis at subchondral bone of MFC and LFC increased time dependently in the early period, however it decreased at the later period. Angiogenic activities of MFC started to increase at 4 weeks and increased to the peak at 6 weeks. After the peak, it took downward turn at 8 weeks and decreased until 12 weeks. Those of LFC took the same tendency and started to increase at 6 weeks, but started to decrease at 8 weeks. On the other hand, the angiogenic activity of cartilage had no changes during study period. This suggested angiogenic activities of subchondral bone increased in the early to moderate phase of OA when cartilage had been degenerated, and activity decreased in the severe OA when cartilage had been lost.

Conclusions: Several previous studies reported that invasion of vascular tissue from the subchondral bone into the cartilage was related with degeneration of articular cartilage. This study indicated that the peak of the angiogenic activities of subchondral bone was in the early stage of OA and presumably followed by the invasion of vascular tissue into the cartilage. This result might implicate new OA treatment in terms of angiogenesis.

Angiogenesis & Synovial Tissue Biology

100 ANGIOGENIC ACTIVITY OF SUBCHONDRAL BONE INCREASES IN THE EARLY STAGE OF OSTEOARTHRITIC KNEE

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