Intra-articular Administration Of Gelatin Hydrogels Incorporating Rapamycin-micelle Reduces Development Of Experimental Osteoarthritis In A Murine Model

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Introduction: Autophagy is a cellular homeostasis mechanism to degrade macromolecules and organelles damaged by various stresses.(1-3) The dysfunction of autophagy has been recently reported to be associated with degenerative diseases and aging.(1,4) We previously reported that autophagy regulates osteoarthritic gene expression of human chondrocytes and the rapamycin, a potent activator of autophagy, plays a preventive role against an inflammatory stress.(5) In addition, a recent study revealed that intraperitoneal injection of rapamycin reduces the development of experimental osteoarthritis (OA) in a murine model.(6) However, the local effect of intra-articular administration of rapamycin on the development of OA remains unknown and needs to be tested considering the side effect of systemic administration of rapamycin. The aim of this study was to investigate the therapeutic effect of intra-articular administration of rapamycin using a murine OA model.

Methods: To release rapamycin in a controlled manner, gelatin hydrogels incorporating rapamycin-micelle was created. Prior to administration, the efficacy of the controlled release of rapamycin from gelatin hydrogels incorporating rapamycin-micelle was examined in vitro. The therapeutic effect of intra-articular administration of rapamycin was examined using a murine OA model in vivo. OA was mechanically induced by destabilizing the medial meniscus under a microscope using knee joints of C57BL/6J mice. Mice (n=56) were divided into 4 groups. Group 1 was used as a control group. Group 2; Treated with single injection of 1μg of rapamycin dissolved in dimethyl sulphoxide. Group 3; Treated with gelatin hydrogels incorporating 100ng of rapamycin-micelle. Group 4; Treated with gelatin hydrogels incorporating 1μg of rapamycin-micelle. Gelatin hydrogels and single injection were administered intra-articulary at the time of the surgery. Mice were sacrificed 10 weeks after surgery. Moreover, to investigate and confirm the long-term effect of gelatin hydrogels incorporating rapamycin-micelle, we created another 2 groups (Control group; n=5, and the group treated with gelatin hydrogels incorporating 1μg of rapamycin-micelle; n=7), and mice were sacrificed 16 weeks after surgery. The efficacy of the controlled release of rapamycin in vivo was examined by immunohistochemistry using an autophagic marker, microtubule-associated protein 1 light chain 3 (LC3) in wild-type mice and by analysis of fluorescence using green fluorescent protein (GFP) fused LC3 in transgenic mice that express GFP-LC3. OA progression was evaluated using the Osteoarthritis Research Society International cartilage OA histopathology grading system. Total number of cells was counted using sections stained with hematoxylin-eosin. To further examine the mechanism of development of OA, RNA was collected directly from articular cartilage of the medial femoral condyle and medial tibial plateau of 3 mice in group 1 and group 4. To investigate the different gene expression pattern, we used DNA microarray analysis and confirmed with real-time PCR. Real-time PCR for matrix metalloproteinase (MMP)-13, MMP-9, CCAAT/enhancer binding protein beta (C/EBPβ), Col2a1, and mammalian target of rapamycin (mTOR) were examined. Immunohistochemistry for MMP-13 was examined.

Results: The in vitro release test showed that rapamycin was partially released in phosphate-buffered saline without collagenase, and in the presence of collagenase, rapamycin was released with time from gelatin hydrogels. Immunohistochemical analysis showed an increased LC3 expression in the mice treated with gelatin hydrogels incorporating rapamycin-micelle 10 weeks after surgery compared with control group. An increased GFP-LC3 signal was observed in GFP-LC3 transgenic mice treated with gelatin hydrogels incorporating rapamycin-micelle compared with the transgenic mice without treatment with rapamycin 10 weeks after surgery. The histological OA score was significantly decreased in both Group 3 and 4 (gelatin hydrogels incorporating 100 ng and 1μg rapamycin-micelle respectively) compared with control mice. OA score was also significantly decreased in Group 2 (single injection of 1μg rapamycin) compared with control mice but significantly high compared with Group 4 (1μg rapamycin-micelle). Histologically articular cartilage was well maintained without obvious progression of OA even 16 weeks after surgery in mice treated with gelatin hydrogels incorporating 1μg rapamycin-micelle. Additionally cellularity was significantly higher in mice treated with gelatin hydrogels incorporating 1μg rapamycin-micelle. Microarray analysis showed a differentially expressed gene patterns between control group and Rapamycin-treated group, and we identified stress-responsive genes.
genes and cytokine-related genes downregulated in the rapamycin-treated mice. In real-time PCR analysis, MMP13, MMP9, C/EBPβ, and mTOR were downregulated while Col2a1 was upregulated in the rapamycin-treated mice. In addition, immunohistochemical analysis showed a decreased MMP-13 level in the rapamycin-treated mice.

**Discussion:** We observed that the intra-articular administration of gelatin hydrogels incorporating rapamycin-micelle suppressed development of OA in the surgically-induced murine OA model. In addition, the delayed OA progression in rapamycin-treated mice was associated with increased expression of an autophagic marker, downregulation of cartilage degrading enzymes and upregulation of cartilage anabolic marker, suggesting that the intra-articular gelatin hydrogels incorporating rapamycin-micelle suppressed OA progression possibly through the downregulating of cartilage-degrading enzymes, stress-responsive genes and cytokine-related genes. Intra-articular gelatin hydrogels incorporating rapamycin-micelle can be a new therapeutic approach for treating patients with OA.

**Significance:** We first showed the local effect of intra-articular administration of rapamycin on the development of OA.

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**References:**

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