Effect Of Inhibiting Mmp13 And Adamts5 Small Interference Rna (sirna) By Intra-articular Injection In A Surgically Induced Osteoarthritis Model Of Mice

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Introduction: In the pathogenesis of osteoarthritis (OA), several proteases including the MMP (matrix metalloproteinase) family and ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family are thought to play a part in the early phase of cartilage degradation. Among them, MMP13 and ADAMTS5 are presumed to be the major players, and inhibition of those proteases has been demonstrated to prevent cartilage degradation in animal osteoarthritic models.

Small interfering RNA (siRNA) silences specific genes by interfering with mRNA translation, and acts to modulate or inhibit specific biological pathways. SiRNA has been proven to be beneficial in knocking down protease effects in vitro, but there are still unsolved problems before applying this method in vivo, due to the molecular instability and difficulty in transfecting siRNAs in vivo. Recently, chemically modulated siRNAs which do not require transfection agents or other additional techniques such as electroporation in transfecting into cells have been developed. These siRNAs can be applied simply to cells with lower cellular toxicity, and is likely to be suitable for in vivo use. Previous studies have demonstrated that the effects of intra-articular injection of ADAMTS5 or MMP13 siRNA into the knee on the delay and attenuation of articular cartilage degeneration. However, the effect of the combined siRNA injection of these two proteases is not clear.

Therefore, the purpose of this study is to examine the effect of administration of chemically modulated MMP13 siRNA or ADAMTS5 siRNA alone, or in combination with these siRNA in a mice OA model.

Methods: OA pathology was surgically induced to nine-week-old male C57/BL6 mice (n=20) by destabilization of the medial meniscus (DMM) method, by transecting the medial meniscotibial ligament under general anesthesia. We used pre-designed Dharmacon Accell siRNAs® (Thermo Scientific, Yokohama, Japan) for Mmp13, ADAMTS5 as well as a non-targeting control. Four different siRNA injections against the DMM model were evaluated. MMP13 siRNA group was injected chemically modulated MMP13 siRNA one week after DMM surgery. ADAMTS5 siRNA group was injected chemically modulated ADAMTS5 siRNA three days after DMM surgery. Combined siRNA group was injected ADAMTS5 siRNA three days after the surgery and MMP13 siRNA four days later. Control siRNA group was injected non-targeting siRNA which does not target to any gene. Each group was injected 1.5nmol/ul siRNAs into the knee joint. Normal group (n=5) did not undergo any surgical induction and intra-articular injection. Histological assessment of articular cartilage at 8 weeks post DMM surgery was conducted to assess the effect of siRNA injections in osteoarthritis progression, according to the OARSI recommendation for OA knee scoring in mice. A Kruskal-Wallis test followed by a Steel-Dwass test was used to statistically analyze the OARSI histology scores between the four groups. P< 0.05 was considered statistically significant.
Results: Fibrillation and loss of Safranin O staining of the cartilage surface were frequently observed in the control siRNA group. Significant improvement was observed in the histological score in all three of the treated group compared to the control siRNA injected group. Especially in combined siRNA group, the score was lower than MMP13 siRNA group (p=0.38) or ADAMTS5 siRNA group (p=0.06). (Fig 1)

Discussion: Injection timing might affect the result but our result indicated combined treatment with MMP13 siRNA and ADAMTS5 siRNA intra-articular injection can be expected to inhibit cartilage degradation in the early phase of OA development compared to treatment MMP13 siRNA or ADAMTS5 siRNA alone.

Significance:

Fig 1. Mean histological grading score (OARSI score) at 8 weeks after DMM surgery of each group (p<0.05)

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