Intradiscal Injection Of Recombinant Human Bone Morphogenetic Protein-7 Significantly Suppressed The Expressin Of Cytokines And Catabolic Enzymes In The Rabbit Anular Puncture Model

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Introduction: Bone morphogenetic protein-7 (BMP-7), also known as osteogenic protein (OP-1), is a member of the TGFβ1 superfamily of proteins involved in the tissue formation, regeneration and proliferation.[1] In vitro studies indicated that BMP-7 was capable of stimulating synthesis of the extracellular matrix by intervertebral disc (IVD) cells.[2] In the in vivo rabbit models of disc degeneration, the anular puncture model [3] and the chondroitinase ABC-induced disc degeneration [4], a single intradiscal injection of BMP-7 resulted in structural restoration (disc height, MRI and histological grades), as well as an increased proteoglycan (PG) content. It is significant to note that this therapeutic intervention induced the restoration of the biomechanical properties of the rabbit discs [5] which is an important point in evaluating the clinical significance of a therapeutic approach. The initial assumption for the mechanism of this therapeutic approach was through the anabolic effects of BMP-7. However, recent in vitro research indicated that BMP-7 has an inhibitory effect on the expression of proinflammatory cytokines.[6] That study has led to our hypothesis that an injection of BMP-7 indeed suppresses cytokine expression in vivo and attenuates the catabolic events occurring after the initiation of disc degeneration.

In the anular puncture model using a 16G needle, cytokine levels have been shown to be elevated for three weeks, followed by a decline at a later time point.[7] In our anular puncture model using an 18G needle, the levels of cytokine expression (interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α)) in the punctured disc were significantly elevated compared to non-punctured discs at 12 weeks after puncture in a preliminary study (data not shown).

The specific purpose of this study was to determine the in vivo effects of rhBMP-7 injection on the expression of cytokines and catabolic enzymes (i.e., a disintegrin-like and metalloprotease with thrombospondin motifs (ADAMTS)4 and ADAMTS5), by the anulus fibrosus (AF) and nucleus pulposus (NP) tissues in the rabbit anular puncture model.

Materials and Methods: The rabbit anular puncture model and rhBMP-7 injection: Eight adolescent New Zealand white rabbits (weighting 3.5-4.5 kg) were used. Under general anesthesia, to induce disc degeneration, the anulus in contiguous discs (L2/3, L3/4, L4/5) was punctured with an 18G needle using the left retroperitoneal approach. Four weeks later, rabbits received an injection of phosphate buffered saline (10 μl PBS group, n=12 discs) or rhBMP-7 (100 μg, in 10 μl PBS, BMP-7 group, n=12 discs) into the center of the NP of previously punctured discs using an open procedure. Rabbits were radiographically monitored for 12 weeks. At the 12-week time point after the injection, the rabbits were sacrificed and the AF and NP separately dissected. AF and NP tissues were stored in RNA later (Qiagen) solution and pulverized after freezing in liquid nitrogen.

Radiological assessments: The percent disc height index (%DHI = (postoperative DHI/preoperative DHI) x100) was calculated as previously described.[8]

Quantitative PCR (q-PCR): The total RNA was isolated from AF and NP tissues and q-PCR was performed using the gene-specific primers for IL-1β, IL-6, 6-ADAMTS4 and ADAMTS5. Standards were made by cloning the PCR products into the Pdrive vector using a PCR cloning kit (Qiagen). GAPDH was used as the internal control.

Statistical Analysis: Two-way repeated ANOVA or the Mann-Whitney test.

Results: Change in DHI: The anulus needle puncture resulted in consistent disc narrowing within four weeks (a 22% decrease from baseline). At 2 weeks after the injections, the disc height in the BMP-7 group had increased towards the pre-puncture level and continued to be higher than that of the PBS group. At 12-weeks post-injection, the DHI of rhBMP-7-injected discs remained significantly higher than that of the PBS-injected discs (PBS vs. rhBMP-7, p<0.001). Cytokine Expression: The comparison between the two treatment groups revealed that rhBMP-7 significantly suppressed the expression of cytokines (IL-1β, IL-6 and TNF-α, p<0.001, Fig. 1) at 12 weeks after the puncture in both the AF and NP tissues.

Aggrecanase Expression: In both AF and NP tissues, the level of ADAMTS4 expression was significantly higher in the PBS group (Fig. 2, top, p<0.001) than that in the BMP-7 group. The levels of ADAMTS5 showed a similar trend that did not achieve significance (Fig. 2, bottom).

Discussion: The study showed that a single injection of rhBMP-7 induced a recovery of disc height and a significantly decreased expression of cytokines (IL-1β, IL-6 and TNF-α) and ADAMTS4 mRNA in the rabbit anular puncture model. The analysis of mRNA has proven to be useful in detecting metabolic changes occurring with anular puncture and BMP-7 injection. Although it is still not clear if a decrease in cytokine levels is the direct effect of BMP-7 on disc cells or an indirect effect from the restoration of disc structure, it can be anticipated that, at a minimum, the stimulation of anabolism by a growth factor also influenced the catabolic pathway. Because these proinflammatory factors can induce a variety of pain markers, such as nerve growth factor, one can hypothesize that this therapeutic approach may have an effect on pain generation. In addition, the inhibition of ADAMTS may suggest that BMP-7 may be able to delay the progression of disc degeneration.
